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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NRC Research Press

ABSTRACTS

Extracting signal from noise: big biodiversity analysis from high-throughput sequence data

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Biodiversity surveys are critical for monitoring environmental health and for the management of natural resources. Genetic methods are gaining broad adoption for surveys of biodiversity and for ongoing biomonitoring. However, bioinformatics methods have not kept pace with the tremendous volumes of data that are generated by high-throughput sequencing platforms. Moreover, many current methods have been developed for microbes but are commonly being applied to data from multicellular organisms due to a lack of alternatives. Here, we present an overview of several ongoing research projects to create new bioinformatics tools to facilitate the rapid and accurate processing of DNA sequence reads from high-throughput sequencers, thus enabling the simultaneous analysis of bulk samples consisting of many species. Specifically, we will present sequencing error models for high-throughput sequencers and results from using machine learning methods to understand which sequence features can be predictive of errors. We will also discuss prospects for using nucleotide and amino acid sequence properties to detect and clean errors in an automated fashion. Our future steps include optimizing methods for clustering DNA sequences into species-like units for biodiversity analysis and developing methods for assigning sequence reads to higher taxonomic categories to unlock functional biological information for diverse bulk samples. These tools are expected to contribute to fundamental research in ecology and biodiversity science as well as to diverse socio-economically important research applications, including the detection of potential vectors or invasive species present in bulk samples.

DNA barcoding of Phlebotomine sandflies (Diptera: Psychodidae) from a leishmaniasis endemic community in Mexico

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Background: Leishmaniasis, a vector-borne disease transmitted to humans through the bite of sandflies, is of public health significance in southeastern Mexico. Active and continuous monitoring of vector species for prediction of potential outbreaks is an important aspect within vector-borne disease control strategies. Thus, correct identification of vector species is paramount to this regard. DNA barcoding in recent years has been proposed as an efficient method of species identification and cataloging. In this study, we employed DNA barcoding as a tool to support sandfly species identification from leishmaniasis-endemic communities of Quintana Roo, Mexico. **Results:** Specimens were collected using CDC light and Shannon traps as part of the health secretariat surveillance program. DNA extraction was carried out using a nondestructive protocol, and identification was performed using taxonomic keys on slide-mounted specimens. Seven sandfly species were identified: *Lutzomyia cruciata*, *Lutzomyia longipalpis*, *Lutzomyia shannoni*, *Lutzomyia deleoni*, *Lutzomyia beltrani*, *Lutzomyia olmeca olmeca*, and *Brumptomyia mesai*. Molecular taxonomic resolution using the DNA barcoding region of the mitochondrial cytochrome c oxidase subunit 1 gene showed to be 100% in congruence with morphological identification. The intraspecific genetic divergence ranged from 0% to 1.24%, while congeneric distances ranged from 13.76% to 20.9%. Maximum intraspecific distances were less than nearest-neighbour (NN) distance. Neighbour-Joining (NJ) analysis also showed that all specimens belonging to the same species grouped together in the tree. **Significance:** The study provides the first sequence records for three species of sandflies and supports the utility of DNA barcoding for species identification of the group in Mexico. We advocate for a detailed study to provide a complete national DNA barcoding reference library for sandflies in Mexico.

Genetic diversity of freshwater fishes of Bangladesh assessed by DNA barcoding

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Background: Bangladesh is very rich in ichthyofaunal diversity and ranked third largest in Asia after China and India, with approximately 251 species of freshwater fish. The ichthyofaunal diversity of the country is under increasing threat from overfishing, habitat destruction, and pollution. DNA barcoding aims to provide an efficient method for species-level identification using the mitochondrial cytochrome COI gene. **Results:** This study represents the first comprehensive molecular assessment of freshwater fishes from Bangladesh. A total of 214 mitochondrial COI barcode sequences were obtained from 137 fish species belonging to 17 orders under 61 families. For all the samples, %G was significantly lower compared to other nucleotides and %GC compared to %AT. Also, a significantly lower GC content was observed in the second and third codon positions compared to the first codon position in all samples. The average Kimura two-parameter (K2P) distances within species, genera, families, and orders were 0.32%, 15.83%, 19.14%, and 25.06%, respectively. DNA barcodes discriminated congeneric species without any confusion, and some new cryptic species have been explored. In addition to generating a reference library for a barcode-based species identification system, phylogenetic relationships among the species have also been attempted. The neighbor-joining tree revealed distinct clusters in concurrence with the taxonomic status of the species. **Significance:** This is the first effort to compile a reference library of DNA barcodes that provides species-level identifications for freshwater fishes of Bangladesh. The study strongly validated the efficiency of COI as an ideal marker for the DNA barcoding of Bangladesh freshwater fishes.

DNA barcoding of marine fishes of Bangladesh

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Background: Bangladesh has vast coastal and marine resources along its south edge as the Bay of Bengal is situated in the south of Bangladesh. Descriptions and information about the marine fishes of Ban-

gladesh are scattered throughout a wide range of publications. An attempt has been made to molecular characterization of marine and coastal fishes of Bangladesh through DNA barcoding, a global bio-identification system for animals. **Results:** A total of 152 species of marine fish covering Carangids, Clupeids, Scombrids, Groupers, Sciaenids, Mackerels, Mullids, Polynemids, Silurids, sharks, and rays representing 13 orders, 91 genera, and 53 families from the Bay of Bengal have been barcoded for the first time using the cytochrome c oxidase I gene (COI) of the mtDNA. The species were represented by multiple specimens, and a total of 304 sequences were generated. The GC content of the teleosts was higher than the sharks and rays (44.2% vs. 41.1%), largely due to a higher GC content of codon position 2 in the former (54.1%). Rays had higher GC than sharks (44% vs. 39.0%), again largely due to higher GC in the 1st codon position in the former (35.0% vs. 20.0%). The average Kimura two-parameter (K2P) distances within species, genera, families, and orders were 0.37%, 9.60%, 13.91%, and 21.00%, respectively. In the neighbour-joining tree for all 304 sequences, three major clusters were apparent: rays, sharks, and teleosts. Species within genera invariably clustered, and generally so did genera within families. **Significance:** Our study provides an example of the usefulness of barcoding for cataloging the diversity of Bangladesh marine fish. Barcode data support the discovery of several new records of species and genera, describe a case of range expansion for a known species, and flagged previously overlooked species.

Species delimitation and phylogeography of African *Parkia* species

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Background: Taxonomic classification within the pantropical mimosoid legume genus *Parkia* has remained controversial for many decades, due to phenotypic polymorphisms and clinal variations. Three parapatric species are recognized in continental Africa for the genus *Parkia* (*P. biglobosa*, *P. bicolor*, and *P. filicoidea*). These species display overlapping ranges between the Sudanian savanna, the Guineo-congolian forest, and the Zambezian miombo. A fourth species, *P. madagascariensis*, is confined to Madagascar. However, there have been much debate and doubt on the exact number of the continental species. The present research aims to assess species boundaries in continental African *Parkia* using genetic and morphological markers. 200 herbarium specimens of *Parkia* species were analyzed for their leaflets characters, capitulum shape, pod width, and morphology. In total, 800 individual plants (including the herbarium specimens) were genotyped using 10 microsatellite markers. Bayesian clustering algorithms implemented in Structure, Tess, and NewHybrids, in combination with phylogenetic and phylogeographical approaches, were applied to identify evolutionary and reproductive units in African *Parkia* species. **Results:** Five differentiated genetic clusters in *Parkia biglobosa* were detected, while *P. bicolor* and *P. filicoidea* each displayed three genetic clusters. *P. bicolor* populations showed higher differentiation from *P. biglobosa* and *P. filicoidea* populations, between which possible interspecific hybrids might exist. Gene pools in *P. biglobosa* are congruent with clinal variations in leaflets sizes in the Sudanian savanna. Likewise, gene pools in *P. bicolor* and *P. filicoidea* followed habitat gradients: the upper Guinean forest, the lower Guinean forest, the Congolian forest, and the Zambezian miombo, some pools being in sympatry. Differentiation and diversity parameters combined with relatedness analyses suggested that genetic clusters within *P. bicolor* and *P. filicoidea* might either represent complexes of cryptic species, or the two species display high phenotypic plasticity. Further analyses using nuclear genes developed for mimosoids are in progress for more resolution to aid species delimitation.

High diversity of dried charales in food markets: a Mexican Barcode of Life network example in the formation of bachelor students

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Background: Charales is a common name used for a dried small fish (5–10 cm) (specially Atherinids) sold in Mexican markets. They are mainly caught in several lakes of the Mexican highlands and the Valley of Mexico. These fishes, due to their nutritional value (as a source of protein and calcium), have been consumed since pre-Hispanic times. Since then, there is a great demand for this product. Only in 2017, more than 8000 tons were produced. Usually, after the catch, they are dried under the sun, so many of the morphological characters are lost. The objective of this project is to identify the species sold as charales by using DNA barcodes. **Results:** More than 7000 dry fish were collected in four markets from the cities of Mexico, Puebla, Oaxaca, and Chetumal. Overall, 18 morphotypes were identified. From them, 163 specimens were selected for DNA barcoding. We obtained 132 positive sequences (81.5% success rate), which corresponded to 7 orders, 13 families, 19 genera, and 21 species (three more than morphotypes). Of these, 42% of the sequences belonged to Atheriniformes, 22% to Cupleiformes, and the final 36% to Perciformes, Cyprinodontiformes, Cichliformes, Siluriformes, and Gobiiformes. *Chirostoma jordani* was the species with the highest number of specimens, followed by species of the family Engraulidae. Twenty-two specimens had no match in BOLD nor GenBank, meaning they have no representatives in current barcode libraries. **Significance:** This study was carried out by bachelor students (fourth semester) and is the first work in which DNA barcodes are used to identify sun-dried fish with successful results. Due to the excessive demand of this product, other fishes different to *Chirostoma* spp. are now being sold as charales, indicating a possible overfishing of them and a stronger pressure on populations of other fish species.

Towards plant barcode 2.0 and its application in environmental and ancient DNA studies

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Background: Identifying barcodes for vascular plants is challenging, and the current ones possess limited applicability in studies of degraded DNA encountered in environmental and ancient samples. To overcome this limitation, we shotgun sequence >1000 species from the Norwegian flora and neighboring regions, and used them as references library for studying ancient environmental DNA and diet through metabarcoding and shotgun sequencing. The latter has the advantage that bias due to PCR is minimized, ancient DNA can be distinguished from modern based on damage pattern, and the full ecosystem can be studied in one experiment. **Results:** We generally assemble full chloroplast and nuclear ribosomal DNA. This reference library combined with metabarcoding has greatly improved our ability to detect species in ancient sediments, and some general patterns are now emerging based on analyses of ~20 sediment records spanning up to 26 000 years. Aquatic vascular plants are exceptionally well represented and often show clear species turnover. The taxa recorded

often indicate a warmer climate than what has been inferred based on pollen records; this is in concordance with macrofossils. In addition, limits of the past northern treelines can be determined more precisely. Applying the method to studies of arctic herbivores reveals that the three dominant plant species of the diet are shared, whereas total diet diversity differs. The advantage of our reference library is even higher when shotgun sequencing environmental samples, as is greatly increased the power to identify taxa. **Significance:** The costs of shotgun sequencing are higher than generating the two formal barcodes by Sanger sequencing. However, applicability of the data is much broader, not only for identifying species, but also for evolutionary studies and the development of targeting specific markers. This large data set, giving 100% resolution, provides a first major dataset for informing the next generation of plant DNA barcodes.

Metabarcoding to trace adulteration in local Greek products

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Background: The global economy of the international trade in herbal medicinal and edible products increases annually by 15%. The rising demand increases the incentive for widespread adulteration and substitution in the raw herbal trade. Substitution with inferior material or unlabeled fillers has been shown in numerous studies. If not contained, adulteration could pose potential health risks to consumers and adversely impact the trade of medicinal plant products in the global market. Current quality and authentication assessment methods rely mainly on morphology and analytical phytochemistry-based methods. However, innovative methods in molecular biology, e.g., DNA-based methods, are becoming more reliable for food authenticity testing with standardized methods and protocols. Here, we use ITS2 metabarcoding to conduct a blind test of the authenticity of 46 herbal mixtures, spice mixes, and medicinal products of Greek origin, of indicated 90 different species of herbs, representing 12 companies. **Results:** Our preliminary results show that 68% of the mixtures contain fillers from the Poaceae family (especially *Bromus* and *Lolium* species), 36% of the mixtures include *Convolvulus arvensis* and *Urtica dioica*, and 30% unexpected *Mentha* sp. In addition, one species of the toxic genus *Euphorbia* was detected. **Significance:** The results of this study highlight quality control deficiencies. Adulteration and substitution of herbal medicinal products is a serious problem that could dilute the effectiveness of otherwise useful remedies.

Assessment of vegetation from soil eDNA metabarcoding: a blast from the past?

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Background: With the increased use of soil environmental DNA (eDNA) metabarcoding for vegetation assessment, there is a strong necessity for a better understanding of the spatio-temporal signal of plant eDNA represented in soil samples. Recent efforts revealed that soil samples capture plant eDNA from highly local vegetation, but yet the temporal resolution of plant eDNA remains unexplored. The lack of historical vegetation records that can be compared to vegetation catalogues derived from metabarcoding have inhibited assessment of the temporal signal of plant eDNA. The availability of detailed vegetation records since 1988 from 100 square metre permanent plots from the Solhomfjell coniferous forest in southern Norway enables the possibility to investigate signals of previous vegetation communities in soil eDNA. Thus, this study aims to address the temporal resolution of plant eDNA in soil samples by cross-checking the taxa retrieved from metabarcoding with the historical records. **Results:** Soil samples from selected plots with vegetation turnover were used for DNA extraction and metabarcoding of plant eDNA. The catalogue of the present flora derived from metabarcoding at each plot was cross-checked and compared with the historical records using matching probabilities. Temporal intervals present in the sample accounting for vegetation structure will be explored using linear mixed models and Procrustes analyses. **Significance:** The results of this study have major implications not only in assessment strategies and sampling design but also in the putative use of soil eDNA metabarcoding for tracking vegetation dynamics.

Construction of customized DNA barcode library facilitates detection of plant insect pests using metabarcoding

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Background: The adoption of DNA barcoding in pest management and regulation has been impeded by (*i*) gaps in taxonomic coverage, (*ii*) discordances between morphospecies and DNA barcodes, and (*iii*) errors in the identification of reference specimens. Unfortunately, these impediments have been perceived as insurmountable because of the need for diverse taxonomic expertise to address them. However, it is possible to bypass some of the challenges by constructing custom libraries that integrate ecological traits, associated with species, and characteristics of errors and discordances. Employment of such an approach serves to address some impediments and improve confidence in use of DNA barcoding in pest management contexts. **Results:** A customized library for insect pests from Canada was developed as a model to test this strategy. A checklist of 511 pest insects reported from Canada was assembled, of which 89% (454) had barcode records on BOLD. These records were enriched with traits, such as plant setting (field crop, greenhouse, forestry), host-plant (species and variety), and geographical origin, mined from the literature and public databases. The taxonomic information was extended with common names, synonyms, and references. The utility of the library, and this approach, was tested by analyzing barcode data generated by metabarcoding insects collected from three greenhouses in Ontario. The comparison identified 43 species of pest insects, 34 matched records on BOLD while nine BINs were new. **Significance:** The study proposes a workflow and a data structure, using Canadian pest insects as a model, that can be employed to construct customized libraries to overcome the three main challenges associated with the application of DNA barcode reference libraries in agricultural or industrial settings. The study also highlights the usefulness of customized library for validation of pest barcode clusters revealed through metabarcoding.

Interspecific dietary compositions and niche overlap of two carnivorous grouper species (Serranidae) from pristine Raja Ampat reefs

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Background: Grouper members, especially *Epinephelus coioides* and *Epinephelus blekeeri*, are carnivorous and play important roles in the coral reef ecosystem. Knowing more about the complexity of their carnivorous diet will provide more information on feeding ecology and the food web in coral reef ecosystems. We used DNA metabarcod-

ing to assess the diet of *Epinephelus* spp., allowing us to improve the data accuracy for ecological function of these species and their prey in the pristine Raja Ampat reefs. **Results:** A total of 5 individuals of each *Epinephelus coioides* and *Epinephelus malabaricus* were purchased from fishermen from Raja Ampat and surrounding reefs. Diet between two these species of groupers was analysed using DNA metabarcoding. Amplicons from the 18S ribosomal RNA, using multiple primer sets, mitochondrial cytochrome oxidase I (COI) gene, and the bacterial 16S ribosomal RNA were sequenced from the gut contents to identify food organisms. Using multiple gene regions will ensure we get the broadest sample. The results presented at the conference are expected to show niche overlap and interspecific dietary compositions between *E. coioides* and *E. malabaricus*. **Significance:** By working iteratively through the food web, we will build a database of species that support commercially and socially important fisheries species, down to the primary producers. This study will also be identifying sites and conditions where the food web is robust enough to support commercial harvesting of targeted species.

Exploring the pelagic ecosystems of the Antarctic with environmental DNA

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Background: The southern Ocean harbours diverse and abundant marine life, distributed along stark latitudinal and depth gradients. Pelagic communities here are highly sensitive to rapid environmental perturbations that make them flagship systems for understanding global climate change. It is imperative to fully appraise ecological structure and resilience to change of these ecosystems if we are to implement successful management to preserve Antarctic resources and ecosystem services. Yet, prospects to expand marine monitoring programs to collect baseline data of ecological trends are stymied by a lack of taxonomic expertise, high expedition costs, and challenging logistics. Environmental DNA techniques have the potential to partly overcome these challenges and could prove a useful complement to existing survey strategies. **Results:** Here, we report on a depth-stratified environmental DNA (eDNA) survey of Antarctic fish and zooplankton communities, conducted as a pioneering study for potential incorporation of eDNA into annual British Antarctic Survey monitoring. Seawater samples were collected during cruise JR16003 of the RRS James Clark Ross at six locations between the Falkland Islands and South Georgia, from six depths between surface and 1000 m per site. Immediately after seawater collection with CTDs, the same sites were trawled using two different aperture nets at the same depth ranges, providing exceptional, contemporaneous comparisons between morphological and molecular methodologies. We present results from both datasets, highlighting limitations of either methodology in the context of long-term monitoring and their strengths in combination.

Technology advancement and DNA barcoding: developing hardware applications for governmental and commercial environments

David Baisch

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A handheld device that can recognize a DNA barcode sequence of any target species could address critical problems facing a tremendous variety of industries ranging from agriculture to customs enforcement. Conservation X Labs is building a device, the DNA BIT, which brings the power of a genetics laboratory to the hands of any user, enabling a rapid, automated DNA test to validate the identify of a wildlife or food product. The value proposition is unlike previous genetic tools—we are focused on democratizing the power of genetics by focusing on the constraints experienced by our potential users and the environment in which they would use it: user-education level, limited or nonexistent power supply, affordability and cost, simplicity, time, scalability, and robustness in the field. We are developing this tool for day-to-day use by nonscientists. Based on frugal design principles and the express needs of customers, the device is designed to support rapid decision making in the environments where they matter—in the field, within the developing world, with the least number of steps possible, and with the highest ease-of-use possible. Our intent through the DNA BIT is to allow governments and private industry to verify the sustainable sourcing of agricultural, marine, or wildlife products, and allow for better management of terrestrial and marine ecosystems. Robust, defensible DNA barcode databases with vouchered specimens will be required to accurately determine species type and provenance for these types of products. Supporting and expanding these databases will build actionable information which can be used to increase supply chain transparency, fight IUU fishing and wildlife trafficking, and help more responsibly manage critical ecosystems on which we all rely. Our vision is to create the tools that put the power of these databases into your hands.

DNA barcoding assessment of species diversity in marine bristle worms (Annelida), integrating barcoding with traditional morphology-based taxonomy

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Background: Assessing species diversity with DNA barcoding of marine polychaete worms in Norwegian waters has provided considerable data and documentation of diversity, lack of knowledge, and demonstrated methodological problems. The marine fauna in Norwegian waters has generally been considered as well known, with a history of species descriptions dating back to Linnaeus. Recent studies using DNA barcoding as a new tool in species taxonomy have indicated a considerably underestimated diversity among polychaetes. About 730 named species are known from the area. Over five years, a large-scale effort aimed at genetically characterising polychaetes has been carried out as part the Norwegian Barcode of Life (NorBOL). The study has spanned a wide range of habitats with variable topography from fjords and coastal waters to deep shelf areas and abyssal waters. **Results:** A total of 4000 specimens of 500 morphospecies have been sequenced, and have yielded 1700 barcodes which group into 700 Barcode Index Numbers (BINs). An average success rate of 50% indicates methodological challenges. Nevertheless, with a varying success rate of 40%–100% and no result for some species, analyses have revealed unknown diversity in all polychaete families represented. From this it is possible to estimate the diversity for each polychaete family. **Significance:** DNA barcoding has contributed to building a reference library for identification of species diversity. Underestimated diversity underpins lack of knowledge of polychaete worms in vast geographic areas. A major challenge for taxonomic work is to assign present species names to BINs. In cases of multiple BINs, the best solution according to basic principles for taxonomic work is to affiliate the name to topotypic material, usually implying a need to sample at type localities. The reference library from DNA barcodes will serve as a backbone when genomic techniques will bring the molecular characterisation of species diversity forward, but also linked to morphological taxonomy.

Insights into long-term biodiversity dynamics assessed through eDNA barcoding and metabarcoding

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Temporal ecological data are essential to study ecological change in ecosystems since their unidirectionality makes them some of the best means to identify causal relationships between drivers and biological responses. Although collecting temporal ecological data is increasingly prominent, only in exceptional cases do data sources cover more than the last 30–40 years. However, environmental DNA (eDNA) can be used as a biomonitoring tool to retrieve historical time series. Therefore, we recently proposed that it is an underused source of temporal biodiversity data that extends hypothesis-driven ecological research to centuries and millennia into the past. Here, we provide an overview of how eDNA can provide new insights into biodiversity dynamics in a barcoding and metabarcoding context. We review existing applications and challenges, and close with an overview on promising directions how eDNA might lead to improved understanding about the ecological consequences of the drivers of long-term biodiversity change.

ACTIAS-WF workflow for a better account of biodiversity

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Background: Despite current efforts, our knowledge of global biodiversity remains incomplete, with most species undescribed. The alarming rate at which species are driven to extinction makes it imperative to have a better account of the biodiversity so we can have an understanding of the whole diversity in the world before it disappears. DNA barcoding, since its introduction, has provided an alternative approach for the identification of described species and discovery of new ones, thus helping to alleviate the taxonomical impediment. Taxonomical data comes basically from two origins: (*i*) species occurrence data coming from a variety of sources (e.g., museum collections, literature records, among others) and (*ii*) DNA sequence data. However, the integration of these two types of data and its accurate placement to delimit species, although crucial, is not always straightforward. **Results:** Here, we present the ACTIAS_WF, a workflow that collects information from several sources and provides the user with tools to make better-informed decisions to assign specimens to a particular Linnean species. Its main objective is to streamline and automate an integrative taxonomy process on the reconciliation of species records coming from either DNA barcodes or occurrences, to assigning them correctly into taxonomic ranks. Developed as part of the ACTIAS project, this computational workflow combines different lines of evidence to document initially the diversity of two sister families of moths, the Saturniidae and Sphingidae. They comprise about 5000 species, and we have assembled a database of ~282 000 occurrence records (i.e., geographical location) from which >77 000 are DNA barcodes. **Significance:** An accurate delimitation of species by integrating these data is essential to study patterns of biodiversity and distribution at a global scale of these insects but also creates the bases for extending the process to other groups of organisms.

Get outside: metabarcoding with the Nanopore MinION

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Background: The coupling of high-throughput sequencing (HTS) with DNA barcoding, commonly known as metabarcoding, has a compelling advantage over traditional approaches for obtaining data on species distributions. Although it has become a more commonly used tool complementing routine biomonitoring techniques, it is often difficult to detect all the species present in a bulk sample. This can—in part—be attributed to the shorter read lengths most HTS instruments generate. Moreover, most HTS platforms are not portable, making in situ field-based sequencing not feasible. Oxford Nanopore sequencing platforms such as the MinION represent an exception to that, and they are also known to provide longer reads, albeit limited by rather high error rates (~12%–15%). **Results:** We used a freshwater mock community of 50 operational taxonomic units (OTU) to test the capacity of the Oxford Nanopore MinION coupled with a rolling circle amplification protocol to provide long-read metabarcoding results. We were able to accurately estimate the diversity of the tested freshwater mock community with an average sequence accuracy of >99% for $1D^2$ sequencing on the nanopore platform. **Significance:** We established a workflow for DNA metabarcoding of freshwater organisms using the Nanopore MinION sequencing platform. We could also show that the high error rates associated with long-read single molecule sequencing can be mitigated by using a rolling circle amplification protocol. Future bioassessment programs will tremendously benefit from portable, highly accurate, species-level metabarcoding, and it appears that we reached a point were cost-effective field-based DNA metabarcoding is possible.

Environmental DNA-based ecotoxin assessments: a Living Lab

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Background: Ecotoxicological impact assessments are traditionally based on morphological observations of a few indicator species and mostly based on laboratory experiments. However, often these laboratory experiments are not empirically relevant as field conditions, which also include species interactions, are far more complex. Moreover, multiple stressors often impact multiple species simultaneously. The use of environmental DNA (eDNA) is a potential game-changer as it allows total community assessments at different trophic levels in field conditions. To gauge the usability of eDNA as early warning for anthropogenic impact assessments, we targeted eDNA of organisms from different trophic levels with metabarcoding in a model system following a multiple stressor setup. **Results:** The experiments were performed in the "Living Lab", a series of artificially created but naturally colonized ditches, which allows for controlled settings in a natural environment. Ditches were challenged to different stressors: insecticides (thiacloprid), excess nutrients, or a combination of both. Water was sampled before and at various time intervals after treatment and analyzed by metabarcoding targeting three different trophic levels: bacterial communities, phytoplankton community, and chironomid communities, as well as general eukaryote biodiversity. Concurrent morphological assessments from the same ditches were performed for comparison. **Significance:** The Living Lab is a perfect model for a functional ecosystem, holding the middle ground between natural and laboratory environment. We demonstrated eDNA techniques to monitor a wide variety of organisms, both prokaryote and eukaryote, to assess the impact of stressors on the ecosystem as a whole. Taxa that have not been traditionally monitored for freshwater quality have become more accessible, now that eDNA techniques have opened up new avenues to detect and identify them. Studies such as these allow for a more reliable and accurate representation of real-life anthropogenically induced community shifts and the identification of vulnerable taxon groups that harbor potential as novel bio-indicators.

DNA metabarcoding of macroinvertebrates in a multiple stressor mesocosm experiment manipulating salinity, fine sediment, and flow velocity

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Background: Stream ecosystems are impacted by multiple stressors worldwide, yet combined effects of multiple stressors on macrozoobenthic communities are still poorly understood. Macroinvertebrates are one of the biological quality elements in stream ecosystem assessments and key taxa in many ecological studies. However, due to their diverse taxonomic composition, associated difficulties with their morphology-based identification, and their sheer abundance, macroinvertebrates are often analyzed with a low taxonomic resolution (i.e., above species level). DNA metabarcoding offers a promising approach to capture this species diversity in the context of multiple stressors more accurately. Here, we used DNA metabarcoding to obtain and evaluate macrozoobenthos diversity in a multiple stressor experiment conducted at a German low-mountain range site. **Results:** In an outdoor experiment manipulating salinity, fine sediment, and flow velocity in a full-factorial design, 99 498 macroinvertebrates were sampled from two microhabitats, i.e., substratum and leaf litter, from 64 mesocosms (8 replicates per treatment). DNA metabarcoding revealed over 400 operational taxonomic units (OTUs) that show a plethora of different response patterns to the experimental manipulation, which were not observed at the family or genus level. For instance, only within the Chironomidae over 120 different OTUs were identified, of which the 35 most common OTUs showed 15 different response patterns. **Significance:** Morphological identification can be insufficient not only when dealing with morphologically difficult groups such as chironomids, but also other taxonomic groups or their juvenile life stages. When pooled to a shallow taxonomic level (i.e., genus or family), response patterns of individual taxa to stressors are masked, potentially leading to false conclusions. On the opposite side, high taxonomic resolution obtained by metabarcoding promises to aid with investigating multiple stressor effects and with assigning ecological traits to operational taxonomic units, which then in return also hold the potential to be used for water quality assessments.

The night shift: improving the conservation assessment of nocturnal flying insects using DNA metabarcoding

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Background: There is evidence for insect population declines worldwide, putting at risk an unknown but potentially very large number of species, and hindering critical ecosystem services. However, except for a few groups such as pollinators, information is still scarce on how anthropogenic drivers affect insect communities. This is partly due to the bewildering diversity of insects and the taxonomic expertise required for species identification. DNA metabarcoding can help offsetting these problems, providing a relatively simple tool for assessing entire insect communities. Here we provide a metabarcoding case study to assess the conservation value of natural (cork oak woodlands and riparian galleries) and agricultural (olive groves and vineyards) habitats for nocturnal flying insects in a Mediterranean mosaic landscape. Insect communities were described from bulk samples collected in July (68 sites) and September (76) 2017 using UV light traps, aiming to quantify community variations in: diversity, composition, and functional traits. **Results:** We detected 1081 operational taxonomic units (OTUs), most of which were Lepidoptera (429), Diptera (244), and Coleoptera (166), but species accumulation curves suggest that many species were missed in each habitat. Richness at each site was consistently lower in vineyards, while olive groves showed comparable richness to cork oak and riparian habitats. There were significant variations in community composition among habitats, with some species occurring exclusively in a single habitat. A functional trait analysis focusing on moths showed that oak and riparian habitats had larger species, while oak habitats had species with more specialised diets. **Significance:** We show the importance of natural habitats for insect conservation in farmed landscapes, but also the key role of extensive land uses such as traditional olive groves. More generally, we show the power of DNA metabarcoding to foster assessments covering simultaneously hundreds to thousands of species, providing much-needed information to include insects in conservation planning and management.

Whole genome shotgun sequencing enables species identification and quantification of pollen species mixtures

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Background: In this study, we tested the ability of a whole genome shotgun (WGS) sequencing method to identify and quantify species in pollen mixtures of known composition. Specifically, we compared WGS sequencing to amplicon-based DNA metabarcoding of ITS2 and rbcL in mixed-species pollen samples. Using the same DNA isolations from constructed pollen mixtures that had been previously analyzed with DNA metabarcoding, we sequenced approximately 200 Mbp for each sample by means of Illumina HiSeq and MiSeq. Taxonomic identifications were based on a k-mer identification method (Kraken) with reference libraries constructed from full genome and short read archive data from the NCBI database. **Results:** We found that WGS was as accurate as rbcL and ITS2 amplicon sequencing in the identification of taxa to the family and genus levels, but less accurate at the species level, and more sensitive to sample complexity. There were also more false positives from WGS relative to amplicon-based methods. We found WGS to provide improved quantification of the proportions of taxa when compared to amplicon sequencing. **Significance:** We found WGS to be a reliable method for taxonomic identification of pollen mixtures. We expect that WGS will become more accurate in the future as the amount of sequence data available for reference libraries increases, and that genomics will become the method of choice over amplicon sequencing for species identification and quantification of mixed pollen samples.

Assessing the spatial and temporal distribution of flower resources for pollinators with DNA metabarcoding

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The assessment of the flower resources used by pollinators can help researchers to understand several aspects of plant–pollinator interactions of significance for the conservation of plants and pollinators and for the ecosystem services they generate. However, there are still major knowledge gaps about the ecology of plant–pollinator interactions such as foraging distances of pollinators, host specificity, and the phenology of flowering and pollinator activity. These factors are challenging and time consuming to assess with observational field assessments. DNA metabarcoding opens a wide range of opportunities for addressing these ecological questions. We present two pilot studies where we have analysed the composition of pollen collected by bees using DNA metabarcoding to assess plant species composition. In the first case, we collected pollen from domestic beehives within Oslo in 2017, in the time period June–September, resulting in 92 pooled samples. In the second case from 2018, we studied pollen loads collected by indi-

vidual bumblebees from various species at altitudes ranging from forest border to the high mountains in the southern part of Norway in order to assess their utilization and preference of various flowering mountain plants during flowering of bilberry (*Vaccinium myrtillus*). We present the protocols for pollen DNA extraction and some preliminary results. In Oslo, we found that the resource use of honey bees is skewed towards typical garden plants, often rich in resources. We also found sharp shifts in pollen composition during the season. However, there is variation among hives in both plant groups and the amount of pollen gathered, indicating strong spatial differences in resource availability in Oslo.

Tagsteady — a metabarcoding library preparation protocol eliminating false assignment of sequences to samples

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Background: Analysis of environmental DNA (eDNA) and community DNA has become a valuable tool in studies of biodiversity, diet, and ecological interactions. Within the field, metabarcoding is the most applied approach as it allows parallel sequencing of taxonomically informative DNA markers from hundreds to thousands of samples. A fundamental assumption of metabarcoding is the reliance on being able to track tagged amplicons back to the samples from which they originated. However, recent studies have identified sequences in metabarcoding sequencing outputs with false combinations of used nucleotide tags, the so-called tag jumps. Tag jumps can be devastating to metabarcoding studies as they can introduce significant levels of incorrect sequence-to-sample assignments, thereby generating false positives and artificially inflating diversity. While the occurrence of tag jumps can be accounted for in the experimental set-up, this is at great expense with regards to both cost and workload. **Results:** We developed and validated the Tagsteady protocol, a protocol for library preparation of pools of tagged amplicons for sequencing on Illumina platforms. The Tagsteady protocol circumvents the two metabarcoding library preparation steps that cause tag jumps. We compared the Tagsteady protocol to protocols that include the two library preparation steps that cause tag jumps and show that the Tagsteady protocol offers efficient, cost-effective tag jump-free generation of metabarcoding data with correct assignment of sequences to samples. **Significance:** To our knowledge, the Tagsteady protocol is the first to ensure tag jump-free library preparation of pools of tagged amplicons for sequencing on Illumina platforms. The efficient, cost-effective and reliable generation of data that the Tagsteady protocol offers is of great value in metabarcoding studies of biodiversity, diet, and ecological interactions. Thereby, the Tagsteady protocol further consolidates and enhances the power of metabarcoding as a tool with which to assess biodiversity and diet.

Arctic BIOSCAN: bridging biodiversity science, DNA barcoding, and Traditional Knowledge in the Canadian Arctic

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Background: The Arctic BIOSCAN project (ARCBIO) is developing the capacity for near real-time DNA-based biodiversity surveillance in the Canadian Arctic, leveraging high-throughput sequencing platforms and recent informatics advances to automate the process of species identification. Its 2-year pilot phase (2018–2020) has two main goals. It will expand the DNA barcode reference library for Arctic organisms, in collaboration with Canada's natural history museums. It will also gather baseline biodiversity data at two sites within the Kitikmeot Region of Nunavut, Canada, representing two bio-climatic zones: Cambridge Bay (middle Arctic tundra) and Kugluktuk (low Arctic tundra), and establish long-term monitoring plots. Surveys will examine representative terrestrial and aquatic habitats in these areas and the major domains of multicellular life. Support from Inuit communities and local experts will aid this research program. An emphasis on country foods sampling will provide insights into wildlife health issues that may affect locally harvested species, as well as northerners and their livelihoods. **Results:** Pilot 2018 surveys in Cambridge Bay deployed an array of standard collecting methods to sample aerial, terrestrial, and aquatic invertebrates; primarily arthropods. They resulted in 586 lots (~65K specimens, over 12K sorted) of invertebrates and 500 specimens of plants. As of January 2019, specimens are undergoing molecular analysis. **Significance:** In order to increase the societal relevance of biodiversity monitoring, ARCBIO will reinforce existing information about Arctic biodiversity by providing the capacity to monitor shifts in ecological communities at a more detailed level. It will also build a new framework for integrating scientific data with Traditional Knowledge sourced through field surveys, research partnerships, and community outreach initiatives. These data will inform public policy in response to ecological and climatic changes, addressing important issues, such as the spread of invasive species, dynamics of wildlife diseases, or shifts in the availability of country foods and other natural resources.

Characterizing diets of mammals from the eastern Mediterranean forest region using DNA metabarcoding

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Identifying food webs and investigating interactions between the various organisms of an ecosystem are necessary steps in evaluating the functioning of the ecosystem and its biological equilibrium. We aim to evaluate the impact of animal wildlife on the long-term sustainability of Lebanon's forests by studying seed dispersal by mammals. Our study will span different seasons to effectively determine the most appropriate time to conduct an ecosystem restoration project. We will use the multi-specific approach of DNA metabarcoding to simultaneously identify both the mammals that occupy Lebanon's forests and their diet during the seasons using noninvasive sampling of scats. While previous techniques such as visual and footprint tracking surveys may work effectively for mammal species identification, they however require intensive work and often lack the resolution needed for diet characterization. Recent DNA-based approaches, like DNA metabarcoding, provide a more accurate method for species identification. These methods have been used in many fields for various applications related to animal diet and biodiversity assessment of species richness. In order to identify food webs in Lebanon's forest ecosystem, we will conduct intensive noninvasive surveys at Ehden Nature Reserve, the most biodiverse nature reserve in the Near East. We have generated a reference library of DNA barcodes for animal and plant species of the region. To date, we have used noninvasive DNA methods to identify 11 mammal species belonging to seven families, and characterized the diets of three species: red fox (*Vulpes vulpes*), wolf

(*Canis lupus*), and beech marten (*Martes foina*). Our study is the first to employ a DNA-based dietary analysis on wildlife in the eastern Mediterranean region where several species are listed by the IUCN as Near Threatened (e.g., *Hyaena hyaena*) or Vulnerable (e.g., *Vormela peregusna*). The results from our work will inform ecosystem management strategies and aid in the conservation of these imperilled species.

DNA metabarcoding for high-density biomonitoring of lakes

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Diatoms are excellent indicators of environmental pressures in aquatic ecosystems. Community assemblages are determined using standardized methodologies based on microscopy which require time and expertise. DNA metabarcoding is a way to avoid these limits and high-throughput sequencing (HTS) offers the opportunity to increase study scale to achieve high-density biomonitoring. The availability of a reliable DNA-barcoding database dedicated to diatoms, such as R-Syst::diatom, is essential for evaluation of ecological status. An overview of DNA-metabarcoding developments for diatoms will be presented together with studies on lake ecosystems based on littoral benthic communities. In particular, a high-density map of the quality of littoral zones of Lake Geneva could be obtained in the program SYNAQUA, as a basis to future preservation or restoration measures. An overall conclusion will be drawn, and perspectives on the use of DNA metabarcoding in lake ecosystems will be given.

eDNA as tool to evaluate and monitor critically endangered flagship wetland species in Upper Guinea Forest protected areas

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Background: Many threatened species exist at low densities and are difficult to detect or monitor due to their elusive and cryptic behaviour. This poses a challenge for conservation managers who require data on the status of these species to inform protected area management practices. We aim to evaluate environmental DNA (eDNA) (species-specific qPCR assays vs. metabarcoding) as a means to detect and monitor three highly endangered species endemic to the wetlands of the Upper Guinea forest zone in West Africa: the Critically Endangered (CR) West African slender-snouted crocodile (*Mecistops cataphractus*), the Endangered (EN) pygmy hippopotamus (*Choeropsis liberiensis*), and the West African dwarf crocodile (*Osteolaemus* sp. aff. *tetraspis*; unevaluated, likely EN). **Results:** We successfully developed species-specific primer and probe pairs for all three target species. Each assay was optimised using both tissue and environmental (water) samples taken from zoological collections in North America and Europe. We here present the results of applying the assay developed for the West African slender-snouted crocodile (*Mecistops cataphractus*) at our field site in a protected area in Cote d'Ivoire, a known stronghold for the species. As expected, we found qPCR to be more sensitive in detecting our target species than DNA metabarcoding. However, we also found crocodilians in general seem to be difficult to detect in situ and that detection did not always match with visual confirmation of our target species. **Significance:** We suggest low detection rates may be due to environmental conditions at our sampling site, nonreptile-specific primers for metabarcoding, or that crocodilians in general do not release as much DNA into water as other species due to their physiological and behavioural traits. We therefore recommend using a combination of traditional methods and eDNA for surveying elusive crocodilian species.

Addressing biological and bioinformatic biases: refining environmental DNA metabarcoding biodiversity inventories on the deep seafloor

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Background: Metabarcoding of environmental DNA (eDNA) enables broader and faster biodiversity assessments, as well as holistic analyses of eukaryote and prokaryote diversity. It is especially useful in environments that are remote and difficult to sample, such as the deep sea, the largest biome on Earth. However, the accurate determination of biodiversity requires an optimal molecular coverage of the living kingdoms that depends on the cocktail of primers used. Moreover, sources of errors in various steps of the metabarcoding workflow (nucleic acid extraction, PCR, sequencing) are suspected to induce overestimations of alpha diversity. Improvements of all steps in the workflow are thus necessary to ensure quality results that meet the standards for accurate baseline data and biomonitoring. **Results:** We addressed technical sampling challenges analyzing bathyal and abyssal sediments using five sets of markers and evaluating samples submitted to several treatments for nucleic acids extraction, including DNA, RNA, and size-selected DNA extracts to limit the representation of short (extracellular) DNA fragments. Finally, we present the improvements that can be achieved using a combination of recent bioinformatic software developments allowing Illumina sequence correction, fine-scale clustering, and post-clustering operational taxonomic unit (OUT) curation. **Significance:** By testing all these tools using mitochondrial COI, as well as 18S and 16S ribosomal DNA markers, we show they can deliver highly improved qualitative data for biodiversity analyses.

Barcoding of Bromeliaceae

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Background: The angiosperm family Bromeliaceae comprises 3906 almost exclusively Neotropical species. The family is characterized by exceptionally high morphological and ecological plasticity, but very low genetic variability. Plants are often vegetatively very similar, which makes determination difficult. Especially in botanical collections this is a problem, when plants are cultivated over many years without flowering. Barcoding is an approach to provide fast and cheap determination; however, the observed low genetic variability in bromeliads causes specific problems. In the scope of the "EvoBoGa" project funded by the German Federal Ministry of Education and Research (BMBF) a number of markers was tested for their suitability for barcoding (nuclear: Agt1, ETS, PHYC; plastid: matK, ycf1). **Results**. The low-copy nuclear marker Agt1 was identified as a potential genetic barcode suitable for identification and applied to taxonomically comprehensive and reliably determined sampling provided by Botanical Gardens, as well as from the private collection of one of the authors (E.M.C.L.). Moreover, an online tool that integrates and links the barcoding data with other scientifically relevant information from bromeliad collections among botanical gardens and herbariums was developed. **Significance**. Easier and more rapid identification improves scientific and public access to and scientific use of living collections.

Biome in a bottle: capturing arthropod and plant diversity using Malaise traps

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Background: Arthropods are the most diverse group of terrestrial organisms and are usually targeted for bio-surveillance. They are collected in large numbers, and Malaise traps are a commonly used capture method. Malaise traps predominantly capture flying arthropods in ethanol. Many of these insects are pollinators and herbivores that feed on plant tissue and (or) pollen. **Results:** Using Malaise traps from Ontario, Canada, we developed a protocol for isolating pollen from Malaise trap ethanol. Ethanol from traps was filtered through a 0.2 μm nitrocellulose membrane. The insects within a trap were given an additional wash with ethanol that was subsequently filtered. Membranes were ground into powder, and the DNA was isolated using a glass fibre-based protocol. DNA from the insects was also isolated. To assess diversity, we used the resulting extracts to amplify cytochrome oxidase I (COI) to track arthropod diversity and an internal transcribed spacer (ITS2) for plant and fungal diversity. **Significance:** This study demonstrates that plant diversity can be captured using Malaise traps where the insects serve as a vector for pollen and fungal spores. Arthropod bulk sampling strategies are also an effective tool for tracking plant and fungal diversity. Such biomes in a bottle increase the utility of bulk sampling strategies for bio-surveillance and ecological research.

Temperate grass allergy season defined by spatio-temporal shifts in airborne pollen communities

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Background: Grass pollen is the world's most harmful outdoor aeroallergen, yet it is not known how airborne assemblages change in time and space. Human sensitivity towards grass pollen varies between species, of which there are over 150 in the UK that flower at different times across the allergy season. However, due to few unique morphological features, grass pollen of different genera cannot be discriminated using traditional observational methods. Currently there is no way of detecting, modelling, or forecasting the atmospheric dispersion of pollen from the biodiversity of grasses, and it is unknown if temporal turnover in species composition match terrestrial flowering or if species richness steadily accumulates over the grass pollen season. Using two complementary DNA barcode markers (rbcL and ITS2), we aim to identify how the taxonomic composition of grass pollen exposure changes across the temperate grass allergy season. **Results:** Here we show that all grass genera display discrete, temporally restricted peaks of incidence which vary with latitude and longitude across Britain. We reveal that the taxonomic composition of airborne grass pollen changes substantially across the grass allergy season, and changes in total grass pollen concentration, measured using traditional observational methods, are the result of many grass species and not a single flowering species of grass. We also demonstrate that local flowering events, with appropriate temporal delays, may be useful for predicting the incidence of particular species of grass pollen in the air. **Significance:** Our results demonstrate how targeted, high-throughput sequencing can be used to understand the biodiversity of airborne pollen communities and fill a substantial knowledge gap that has persisted over the past 50 years of aerobiology research. By developing more refined aeroallergen profiling, we anticipate that our findings will facilitate the exploration of links between taxon-specific exposure of harmful grass pollen and disease, with concomitant socio-economic benefits.

Improved methods of marine monitoring: combining genetic and acoustic approaches to reveal pelagic fish biodiversity

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Background: Standardised monitoring of fisheries stocks is vital for their effective management. The use of hydro-acoustic sounders to identify schools of fish has been an established survey method for decades. While the identity of fish species detected is indirectly inferred from the frequency response, backscatter strength, and the location of fish in the water column, validation is typically performed by means of trawling activities. However, these are time-consuming and therefore expensive and require special equipment that cannot be conducted from all acoustic platforms. In addition, the resulting catch composition is often affected by species and age-specific catchability, which is difficult to quantify and not representative of the species composition of the acoustically observed targets. Environmental DNA (eDNA) could become a tool that revolutionises standardised surveys with its power to verify fish species and population composition. **Results:** This study investigates the potential for using DNA as a qualitative and quantitative tool for the assessment of marine pelagic communities. To investigate these possibilities, we collected DNA seawater samples during the PELTIC 2018 survey on the Cefas Endeavour around the southwest coast of the UK, from surface water during trawls, and from a CTD Rosette at pre-determined sampling stations. The PELTIC survey is used to map and quantify economically, as well as ecologically, important pelagic fish species, including sardine (*Sardina pilchardus*), sprat (*Sprattus sprattus*), European anchovy (*Engraulis encrasicolus*), Atlantic herring (*Clupea harengus*), and Atlantic mackerel (*Scomber scombrus*). DNA metabarcoding of 12S mitochondrial amplicons is being used to target these, and other fish species; then the spatial faunal composition of the species data will be compared to the hydro-acoustic and trawl data. **Significance:** The patterns obtained will be examined to achieve a better understanding of pelagic communities around the southwest of Britain and to devise improved approaches for the monitoring of marine biodiversity.

Making good on the promise of molecular monitoring

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Molecular methods have long promised to revolutionise biodiversity monitoring worldwide, and have now reached the point where environmental managers and policy makers want to use them. For instance, the UK government has adopted environmental DNA (eDNA) methods for detection of individual protected species and is now starting to use eDNA metabarcoding for routine monitoring of fish communities. However, translation of protocols from a research environment into an operational, commercial, or regulatory one is not trivial, requiring continuity of service, standardisation of methods, robust QC procedures, and mechanisms that enable nonspecialist end-users to navigate and evaluate the services offered. Based on my experiences in (*i*) running a business that specialises in delivering

DNA-based monitoring services and (*ii*) leading the development of the first European Standard for eDNA applications, I reflect on the challenges and opportunities associated with real-world uptake of molecular monitoring tools and highlight the need for close collaboration between researchers, policy-makers, and service providers to achieve the revolution promised by new technologies. I draw on examples from the UK to the Amazon rainforest to demonstrate the power of DNA-based monitoring in real-world monitoring applications.

DNA barcoding reveals cryptic diversity and genetic connectivity in the deep-sea annelids across the Greenland–Scotland Ridge

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Background: The Greenland–Scotland Ridge (GSR) acts as a topographical barrier separating the Nordic Seas from the North Atlantic, with a maximum threshold depth of 840 m in the Faroe Bank Channel. The GSR has been shown to affect the distribution of various deep-sea benthic organisms such as crustaceans and mollusks. Annelids are very prone to cryptic speciation especially along the depth gradient, and numerous examples of species complexes comprising multiple genetic lineages have been reported from the North Atlantic and the Arctic. We aim to estimate the role of GSR in genetic connectivity in the annelid fauna between the deep Nordic basins and the deep areas in the Northern Atlantic south to GSR and to identify the degree of bias in assessing the distribution of annelid morpho-species in comparison to species delimited based on genetic information. **Results:** The present study is based on deep-sea samples obtained during MAREANO, HAUSGARTEN, and IceAge 1 and 2 expeditions. We assess morphological and genetic diversity in seven genera of annelids, representing the families Ampharetidae, Lumbrineridae, Opheliidae, Scalibregmatidae, Sphaerodoridae, Spionidae, and Terebellidae. Species are delimited based on single or multiple loci analyses of mitochondrial (COI, 16S rDNA) and nuclear (ITS, 28S rDNA) markers. In each studied genus of the deep-sea annelids, multiple cryptic lineages are detected after applying molecular species delimitation tools. The distribution of several molecular-delimited species inhabiting bathyal and abyssal depths across GSR is confirmed, posing a question on the mechanisms of the deep-sea species dispersal across topographic barriers. **Significance:** Identification of species based on exclusively morphological data significantly affect correct assessment of geographical and vertical ranges in the deep-sea annelid species. DNA barcodes based on COI sequences are, in most cases, effective indicators of putative species; however, additional nuclear markers are needed for robust species delimitation analysis.

DNA barcoding and the study of incipient speciation in Neotropical birds

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Background: The Neotropics constitute the most biodiverse region of the world, including over 3000 bird species. The complex patterns of diversification of Neotropical birds, however, have been much less studied than in the Nearctic and the Palearctic, which is particularly true for the temperate south of South America. DNA barcoding of southern Neotropical birds showed that several species possess divergent intraspecific mitochondrial lineages, which constitute excellent cases to study the speciation process in the region. We are studying *Vanellus chilensis* (Southern Lapwing), *Thamnophilus ruficapillus* (Rufous-capped Antshrike), and *Pipraeidea bonariensis* (Blue-and-yellow Tanager). These species represent different avian radiations and have contrasting distributions and habitat preferences, thus allowing for the study of the different factors of diversification that have been proposed for the Neotropics. **Results:** Mitochondrial (COI + cytochrome b) patterns of divergence suggest that different factors acted in each species: the glacial cycles in *V. chilensis* (its main split is between Patagonia and the rest of South America), the Andes in the other two species (both show deep sequence differences along the Andes), and also the open vegetation corridor in *T. ruficapillus* (deep divergence between the Atlantic Forest and the Andes). These mitochondrial patterns are currently being studied in more depth using a genomic approach (ddradseq) to better assess the diversification history within each species, the level of gene flow among lineages, and the role of the diversification factors mentioned above. We are also studying colouration and vocalizations, which could shed light on the process of phenotypic diversification in these taxa and help to elucidate whether some of these intraspecific lineages deserve species status. **Significance:** This study is increasing our knowledge about the patterns of evolution in the temperate Neotropics and shows that combining DNA barcoding scans with additional sources of genetic/genomic, morphological, and behavioural information allows performing sound evolutionary studies.

DNA metabarcoding in the past, present, and future

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Background: Modern or ancient DNA isolated and characterised from a variety of biological substrates including sediment and water is collectively referred to as environmental DNA (eDNA). DNA is shed into the environment from a variety of biological secretory processes, leaving a genetic footprint that acts as a lens into species composition. When combined with next-generation sequencing (NGS) and metabarcoding, eDNA can provide a wealth of information for studies of biodiversity, food web dynamics, diet analysis, invasive species monitoring, disturbance gradients, and extinctions. Metabarcoding eDNA has become feasible only because it is now possible to simultaneously sequence millions of copies of DNA from complex multi-species environmental samples. **Results and Discussion:** This plenary will present data and discuss how DNA metabarcoding approaches have been used to profile (*i*) fossil assemblages from across the globe using ancient DNA preserved at archaeological and paleontological sites; (*ii*) herbal medicines, how metabarcoding can be used to validate species composition; and (*iii*) how temporal and spatial eDNA surveys are transforming how we monitor and explore our marine environments. The sensitivity and power of metabarcoding also comes with risks including false positive/negatives and contamination. This presentation will discuss the importance of each step in the metabarcoding workflow and the rationale behind safeguards that should be considered as the field of eDNA metabarcoding matures at a rapid rate and seeks to gain acceptance across a variety of applications.

Identification of fish species of sushi products in Hong Kong

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Background: Sushi is a popular food product in Hong Kong and around the world. It has been found susceptible to fraudulent substitution or mislabelling in various countries in North America, Europe, and Asia. In Hong Kong, the hygiene aspect of sushi products is tightly

regulated, with regular evaluation of bacterial content. However, less concern is put on the authenticity and labelling of sushi products. **Results:** The accuracy of the English and (or) Chinese names on the product labels was also evaluated based on the common names of the identified species shown in FishBase (for English labels) and The Fish Database of Taiwan (for Chinese labels). Accuracy of local common names (in Chinese) was assessed individually. Ninety-three pieces of sushi, with 85 fish samples and 43 roe samples, were collected from restaurants and other retailers for 16S rRNA gene sequencing. All samples were successfully sequenced. Species identification was performed by BLAST and phylogenetic clustering. The molecular results were then used to verify the information declared at purchase. Out of the 128 samples, 96 samples were labelled in English and 107 samples in Chinese. The overall rate of mislabelling in English was 26.0% (25/96) and that in Chinese was 17.8% (19/107), reflecting a moderate level of mislabelling. The English labels phonetically translated from Japanese had a high rate of mislabelling (40%). The most commonly mislabelled sushi type was herring roe of herring sushi, most of which was identified as *Mallotus villosus* (capelin). **Significance:** The present study represents a first DNA-based survey for the identification of fish and fish roes used in sushi products commonly available in Hong Kong.

Molecular analysis of Kemp's ridleys (*Lepidochelys kempii*) from Mexico

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Background: The Kemp's ridley turtle (*Lepidochelys kempii*) is one of six sea turtles that nest in Mexico. Except for a tiny population in the southeast coast of the USA, the species nests exclusively in Mexico. The objective of this study was to provide the first DNA barcodes of Kemp's ridleys nesting in Mexico and evaluate the intraspecific variation in the COI marker for this species. **Results:** Sequences from a total of 99 individuals of *L. kempii* were analyzed. Ninety-two sequences were obtained from tissue samples of dead turtle hatchings or peripheral blood from females nesting in Rancho Nuevo, Aldama, Tamaulipas, Mexico; seven additional sequences were downloaded from BOLD. In total, five different haplotypes were observed. Most sequences (95) belonged to the same haplotype, while four haplotypes were singletons. All haplotypes were found in the same locality, the sanctuary of Rancho Nuevo, Mexico. The average genetic distance within this species had a value of zero. **Significance:** Data on intraspecific genetic variation is important for species identification, population genetics, and conservation biology. This work provides COI sequences for *Lepidochelys kempii* and describes new haplotypes for this species.

Molecular analysis of East Pacific green turtles that nest in Mexico

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Background: *Chelonia mydas* is one of the most studied species of marine reptiles and is according to IUCN and CITES is currently listed as Threatened. It has a global distribution in subtropical and tropical areas; the eastern Pacific population is also known under the subspecies *Chelonia mydas agassizii*. In Mexico, turtles nest from the coast of Michoacán to the Revillagigedo Islands, while in the Atlantic they nest in the Gulf of Mexico and the Caribbean. This work aims to determine if there is variation in the COI gene that differentiates populations in the East Pacific from those in the Atlantic in Mexico. **Results:** Among 60 analyzed sequences, 35 were downloaded from BOLD, and 25 samples came from two Mexican locations: The Gulf of Mexico and Pacific Ocean. COI sequencing yielded a 625 bp long fragment for 60 individuals of *C. mydas*. A total of 10 bp (1.6%) were polymorphic, and these defined 10 haplotypes. The phylogenetic tree construction was performed using maximum likelihood (ML) under the K2P nucleotide substitution model, with 10 000 bootstrap replicates. Clustering analysis and haplotype networks strongly suggest two distinct, major lineages, one from the Atlantic and one from the Pacific Ocean. **Significance:** This work provides information about variation in COI for green turtles that nest in Mexico and is relevant to conservation of this species. It also provides additional reference barcodes for marine turtles, in particular from Mexico.

Paleogenomic annotation of historical *Cinchona* bark samples across time and space

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Background: With the advent of high-throughput sequencing techniques of ancient DNA (paleogenomics), new opportunities for exploring and using natural history collections have emerged. *Cinchona* bark, the source of quinine for treatment of malaria, is an outstanding time-referenced model system. We are using both modern samples across their geographical range, as well as unrivalled historical collections of about 1000 specimens documenting 150 years of collecting and breeding experiments including samples from forests that no longer exist as well as the chemically annotated collections of Howard & Sons. However, the botanical and geographical origins of the historical samples were poorly recorded, preventing us from utilizing these extensive collections until now. This project is generating paleogenomic data from *Cinchona* bark collections to resolve the phylogeny of the tribe Cinchoneae, potentially determining the proportion of adulterated specimens in the historic collections. A modern phylogeny would also provide critical links for determining changes in the distribution of *Cinchona* forests over 200 years, shedding light on evolutionary processes over short periods. Additionally, Howard & Sons chemically annotated hundreds of these historical samples, and they are now undergoing HPLC analyses to trace the alkaloid content dynamics over time. **Results:** We have been able to extract DNA from historic *Cinchona* samples that are around 200 years old from the Economic Botany collection at Kew Gardens. The modified (PVP 1%) phenol-chloroform methodology by Wagner et al. (2018) turned out to be the best DNA extracting protocol, yielding DNA concentrations of 2–5 ng/μL. **Significance:** Museum collections are a valuable source of biodiversity for evolution, authentication, drug discovery, and other studies.

Clarifying the taxonomic status of *Mastigodiaptomus albuquerquensis* using DNA barcodes and morphological analysis of Nearctic and Neotropical specimens

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Background: The freshwater calanoid *Mastigodiaptomus* is a genus with high richness in the Americas, actually composed of 13 species—

most species are recorded in Mexico, and five species apparently are endemic to small areas of Mexico, Central America, and the Caribbean. *Mastigodiaptomus albuquerquensis* has been considered as a common, widely distributed species ranging from the southern USA to Central America. This affirmation was made under the assumption that the species was easily identified by a notable butterfly-like sclerotization on the basis of the right fifth leg of males. However, in this work, the taxonomic status of the species is clarified using modern, integrative methods based on the COI gene as a DNA marker, plus microstructural analysis (based on SEM and light microscopy). **Results:** The analysis allowed the identification of at least three cryptic or pseudocryptic species from the *Mastigodiaptomus* species complex: *M. patzcuarensis*, *M.* cf. *albuquerquensis* (from Central Plateau of Mexico), and *M. cuneatus* (from northwestern Mexico). In addition, morphological differences observed among populations throughout the original distributional range have led to the description of several related species, such as *M. suarezmoralesi* and *M. siankaanensis* (from southeastern Mexico). Finally, a genetic distance of 2%–6.54% (±0.003%) was found between these species.

eDNA in the field: identifying factors that influence the detection of benthic macroinvertebrates using environmental DNA in northern freshwater habitats

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Background: Environmental DNA (eDNA) analysis has the potential to provide insight on a broad range of taxa with less sampling effort than traditional methods. Because caddisflies (Trichoptera) are widely used as bioindicators due to their sensitivity to pollution, they would benefit from a more standardized sampling method like eDNA surveys. During August 2018 we sampled 22 coastal ponds in Churchill, Manitoba, for a particularly widespread and abundant species (*Philarctus bergrothi*) using both eDNA and traditional sampling methods (i.e., kick-netting and rock washing) to determine which factors most affect the detection of macroinvertebrates using eDNA in northern freshwater ponds. Species-specific primer and probe sets were designed, and targeted real-time quantitative PCR (qPCR) tests were run on the extracted eDNA sample from each pond. We were able to obtain presence/absence data at a field station shortly after sampling (in under 3 h) using a portable setup. **Results:** Our preliminary analyses indicate that qPCR (with 6 replicates per pond) can be used to detect reliably our target species at sites where it is known to occur. Also, we found a positive relationship between abundance of target organisms, conductivity of a pond and positive detection rates, which could indicate a better preservation of eDNA in saline environments. We will also review the experimental setup, which allowed us to obtain results without having to bring samples back to a central laboratory. **Significance:** With this work we are gaining a better understanding of what impacts species detection with eDNA. Our testing of methods is also key in making arthropod sampling faster, more accessible, and more cost effective. This knowledge is imperative to develop standardized, validated eDNA assays that will drastically enhance our ability to detect invasive species, track shifts in geographic distributions due to climate change, and survey a greater portion of Arctic biodiversity.

Characterization of the microbiome from high-altitude permafrost-affected soil

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Background: High-altitude permafrost has not received much attention and biological study as has been devoted to Arctic and Antarctic permafrost. To date, metagenomic studies focused on the 16S rRNA gene as a phylogenetic marker enable us to explore the microbial compositions in a range of sample types while bypassing the need for a culturing process, allowing the characterization of a vast array of microorganisms never previously isolated in culture. Microbial responses to climate change and, in particular, thawing of permafrost soils are not yet well understood, despite the threat of microbial contribution to positive feedback of carbon flux through aerobic and anerobic respiration of microbes sustained into the sub-zero temperature range. Hitherto, several studies have tried to understand the microbial community dynamics in permafrost due to changing environmental conditions. In this study we attempt to characterize the microbiome of high-altitude permafrost-affected soil from the Changthang region of Ladakh. **Results:** Phylogenetic analysis of sequences obtained from the permafrost-affected soil indicates that population of bacteria inhibits these soils. Various bacterial phylum, namely Proteobacteria, Bacteroidetes, Acidobacteria, Actinobacteria, Planctomycetes, Nitrospirae, Chloroflexi, Elusimicrobia, Fibrobacteres, etc., are thriving in it. Among them, phylum Proteobacteria dominates the permafrost soil microflora, which indicates that they are the core component of the permafrost soil microbiome. Furthermore, the bioinformatic analysis of the sequences reveals that three major phyla of archaea are dominant in the soil samples, i.e., phyla Crenarchaeota, Euryarchaeota, and Parvarchaeota. Phylum Euryarchaeota includes members from three important orders, i.e., Methanobacteriales, Methanomicrobiales, and Methanosarcinales. All of these are methanogenic in nature. **Significance:** Our result suggested that the extreme terrestrial environments are excellent niches for specialized microorganisms belonging to the domains of Bacteria and Archaea. As the permafrost thaws, the microbiome thriving in this soil has the potential to degrade the sequestered organic carbon in the permafrost of this region.

Development of multiplex PNA-FMCA for accurate and rapid analysis of seven species of eels

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Background: In Northeast Asia, eels are recognized as healthy food and are traded at a high price. Occasionally, merchants deceive origin or species because they are difficult to distinguish morphologically. Consequently, a genetic analysis method using peptide nucleic acids (PNA) probes was developed to determine the exact origin and species. **Results:** The nucleotide sequences of the mitochondrial cytochrome oxidase subunit 1 (COI) gene of eel species were analyzed for the identification of seven species. Probe-based fluorescence melting curve analysis (FMCA) is a powerful tool for mutation detection based on melting temperature generated by thermal denaturation of the probe-target hybrid. The PNA probes have several advantageous features, such as easiness of probe design and modification, without false negative. In this study we have developed a molecular method base on real-time polymerase chain reaction (real-time PCR) technology for the rapid identification of seven eel species. Seven PNA probes were designed to identify *Anguilla anguilla*, *A. japonica*, *A. bicolor*, *A. bicolor pacifica*, *A. marmorata*, *A. mossambica*, and *A. rostrata*. Seven PNA probes in two reactions were positioned in cover the COI gene region. PNA-FMCA with color multiplexing was used for the identification of eel species. **Significance:** The PNA-FMCA system can distinguish target species from others in an efficient and high-throughput manner and can be applied to species identification of eel. The dual-labeled PNA probes offered advances of improved flexibility in probe design, which would provide various applications in genotyping of wide range of spectra. Therefore, it is possible to identify eel of seven species accurately, rapidly, and simultaneously using the PNA-FMCA system.

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*

represent such a case with similar morphologies, where the former is considered as a good indicator of highly eutrophic conditions, and the latter has been described as oligotrophic. A similar challenge exists between *N. palea* var. *tenuirostris* and *N. gracilis*, where the latter is known to be more sensitive to organic pollution. Standard DNA barcoding markers have been proposed and used for DNA metabarcoding of diatom communities, including the V4 subregion of nuclear 18S rDNA, chloroplast rbcL gene, and mitochondrial cox1 gene. A correlation between molecular and morphological data was obtained using 18S rRNA and rbcL markers; however, discrepancies in species-level identification were reported for certain taxa. Recent genomic analyses suggest that diatoms and some relatives originate from a serial secondary endosymbiosis event involving both green and red algae and a heterotrophic host. Moreover, frequent biparental inheritance of chloroplasts and autopolyploid formation were reported for some taxa. Thus, considering the complex evolutionary history and inheritance patterns in diatoms, a multilocus phylogenetic approach covering all three genomic compartments is needed to resolve taxonomically challenging cases such as the *Nitzschia palea* species complex. In this study, we will prepare monoclonal cultures of target diatom taxa in collaboration with the Alverson Lab (University of Arkansas) and use transcriptomics to develop innovative genomic markers which can delineate species boundaries to ultimately improve water quality assessment.

Dietary versatility of coral reef fishes in response to habitat degradation

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Background: The decline of coral reef habitat directly affects dietary patterns of benthic feeding fishes. The degree of dependence on particular food types determines each species' sensitivity and adaptive capacity. While generalist feeders are expected to be less vulnerable to changes in prey availability than specialist feeders, the extent to which species manage to expand or switch diet as a behavioural response to environmental changes is poorly known. **Results:** We use a DNA-based approach (metabarcoding) to link changes in fish diet with habitat quality across the Bahia Almirante of Bocas del Toro, Panama. Metabarcoding of gut contents of two invertebrate-feeding fish species representing different feeding strategies (*Chaetodon capistratus*, a browser and *Hypoplectrus puella*, an active predator) revealed dietary responses for both species to variation in coral cover. However, the response was much more pronounced for the browsing species. Our results indicate a behavioural switch from browsing towards active predation when adjusting to degraded environments that suggests negative consequences on fish fitness. **Significance:** This study refines our understanding of mechanisms of dietary niche alteration in coral reef fishes that are impacted by the degradation of their habitat.

Namib desert Collembola are deeply divergent and spatially isolated

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Background: Four springtail (Collembola) species have to date been reported from the Gravel Plains of the Namib Desert (14.5°E, 22.5°S to 16.5°E, 24°S), although no specific taxonomic keys are currently available. We provide the first sequences for Namib Desert springtails and assess spatial variation in the context of dispersal and population connectivity. **Results:** We obtained COI sequences from 341 springtail specimens, 175 of which were unique haplotypes. Generalized Mixed Yule Coalescent analyses identified 30 putative species, with up to 28% sequence divergence (uncorrected p-distance). The spatial distribution of genetic variants was disjunct, with 97% of haplotypes and 70% of putative species found only at single sites. **Significance:** Dispersal in this region, although rare, may be facilitated by environmental events such as prevailing onshore winds or occasional flow of rain water to the coast. We conclude that the high genetic diversity we observed indicates the presence of ancient springtail lineages, patchy distribution of suitable habitats, and limited dispersal/gene flow among habitable locations.

Unlocking environmental genomics for routine biomonitoring with machine learning

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Aquatic environments provide numerous invaluable ecosystem services. Due to the ever-growing anthropogenic pressures on these habitats, their ability to sustain biological communities and services can be severely impacted. Because there is a trade-off between acceptable environmental impact of ecosystem exploitation and socio-economic benefits, international regulations for sustainable industrial development with minimal environmental impacts are in place worldwide. The backbone of such monitoring programs for aquatic habitats is the biological component. As opposed to physico-chemical monitoring techniques, biological indicators provide a cumulative measure of the disturbances that occurred in a given environment. Until recently, routine biomonitoring relied mainly on the morphological identification of bioindicators taxa to compute biotic indices (BIs), which is time and expertise demanding. Environmental genomics, especially the high-throughput amplicon sequencing of multiple species in environmental DNA (eDNA) metabarcoding has revolutionized our understanding of biological communities. Yet, its use for routine biomonitoring is still considered a futuristic ideal. Until recently, these molecular tools were mainly used as a replacement for the burdensome morphological identification to screen known morphologically distinguishable bioindicator taxa. However, sampling large-size organisms with environmental DNA suffers from a strong sampling effect, which seriously hampers its usefulness to compute biotic indices that largely depend on taxa abundance. Furthermore, eDNA datasets are largely dominated by inconspicuous, microbial, or meiofaunal taxa, for which reference sequences in public database and ecological knowledge remain scarse. To overcome these limitations, we used a Supervised Machine Learning (SML) approach to directly predict the BIs values from metabarcoding data, regardless of the taxonomic affiliations of the sequences. We showed that such an approach is accurate and that it outperforms current taxonomy-based metabarcoding approaches for biomonitoring. We argue that adopting SML into routine biomonitoring programs will unlock the environmental genomics potential.

Phylogeny and barcoding with complete species coverage resolve relationships in the squid genus *Lolliguncula* Steenstrup, 1881

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Background: Squid of the family Loliginidae from the new world have been the subject of a number of phylogeographic and regional

analyses in recent years, but a number of important species have been missing from these analyses. Notably, they did not include *Lolliguncula argus*, a species that has recently had new samples registered from northwestern Mexico. Systematic relationships within the genus and the relative position of *L. argus* can therefore now be resolved. The new samples of *L. argus* have been included in a multigene phylogeny including standard barcode sequences, and species were delimited using both threshold and coalescent methods. **Results:** Based on COI data, *L. argus* and *L. diomedeae* show very little molecular divergence. Species delimitation results vary by method and choice of threshold. Most methods indicate that these are not distinct species. However, lower thresholds do indicate them as separate. As morphological differences do apparently separate these species, the overall output suggests recent speciation. A time-calibrated phylogeny places this speciation event at 2.5 mya, possibly related to recent changes in currents and connectivity in the eastern tropical Pacific as a result of the formation of the Gulf of California and the subsequent closing of the Isthmus of Panama. The remaining species are confirmed to represent a monophyletic group and the valid species: *L. panamensis* ((*L. argus* + L. *diomedeae*) + (*L. brevis* N Atlantic + *L. brevis* S Atlantic)). **Significance:** The complete phylogeny provides confirmation of the importance of considering fisheries stocks of *Lolliguncula* from the Gulf of California as a distinct evolutionary unit, despite the recent divergence, and provides the basis for an improved time-calibrated phylogeny and biogeographical model for these and other new world loliginid squid.

Identification of plant species in mixed-seed samples using high-throughput sequencing technology and DNA barcodes

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Background: DNA barcoding has been proven to efficiently discriminate species from their relatives as a means for identification when morphological characteristics are insufficient. For example, species discrimination of closely related species by seed analysts can be limited to the genus or family level due to the lack of distinctive morphological characteristics. Furthermore, this analysis is arduous as it requires manually searching the seed sample for different taxonomic groups followed by a morphological analysis for species identification. The process can be lengthy due to the number of seeds in the samples but also for samples containing seeds of irregular shapes, small seeds, or mixed seeds like wild flower seeds. The goal of this study is to test if DNA metabarcoding can be used to identify and discriminate plant species from their relatives in mixed-seed samples. **Results:** A workflow combining HTS and bioinformatics was developed to identify specific plant species present in a known mixture of wild flower seed sample. Briefly, samples of mixed wild flower seeds were screened by a seed analyst using traditional morphology-based methods. Representatives of each taxonomic groups found in these samples were subsequently DNA barcoded to serve as reference. DNA extracted from the seed mixture was amplified for specific barcode regions and then sequenced using HTS. Species in the mixed-seed sample were then identified using the reference DNA barcodes. Comparative results from the new HTS-based workflow and the morphological analysis will be reported and discussed. **Significance:** DNA metabarcoding allows a timely and simultaneous identification of multiple plant species present in mixed-seed samples with higher specificity and high sensitivity. Ultimately, this workflow will be adapted for the identification of weed seeds mixed in imported grain or crop seeds, which is one of the main pathways for the unintentional introduction of invasive plant species.

Assessing lotic biodiversity via eDNA analyses across heterogeneous land use types: patterns and processes

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Background: Understanding the impacts of environmental change on biodiversity and ecosystem function requires comprehensive knowledge of ecology, environmental pressures, and land use. The analysis of environmental DNA (eDNA, i.e., shed cells or free DNA from organisms as they pass through an environment, or die and decay) provides an opportunity to measure biodiversity in space and time at unprecedented scales. However, understanding how to effectively analyse eDNA and how sources of eDNA relate to biodiversity in ecologically and socio-economically important river ecosystems is yet to be resolved. The UK NERC Highlight Topic Grant "LOFRESH" aims to understand the spatial, temporal, and ecological relevance of temperate lotic eDNA in relation to functional community biodiversity. Here, using unique experimental semi-natural stream ecosystems, we will appraise the efficacy of using qPCR, metabarcoding, and shotgun sequencing in detecting the biodiversity of macroinvertebrates and fish fauna. Further, we aim to assess linkages between community composition of fish, macroinvertebrates (referenced via morphological assessments), diatoms, and broader biodiversity, as measured by eDNA analysis from approximately 30 catchments associated with different land use types. **Results:** We will explore if metabarcoding or shotgun sequencing can be used as appropriate "catch all" solutions compared to targeted qPCR biodiversity detection approaches. A range of environmental and ecological variables will also be modelled to explore the defining factors shaping lotic biodiversity in relation to physicochemical parameters and land-use factors at a range of spatial scales (e.g., within catchment and national scale). **Significance:** The current state-of-the-art of lotic eDNA analysis focuses on the accuracy of species detection. Here, we aim to explore linkages across the terrestrial-riparian interface and the biodiversity of a breadth of microbial eukaryotes, macroinvertebrates, and vertebrates to explore how eDNA traces reflect ecologically relevant assemblages and offer insights into the functional consequences of how land-use pressures affect biotic communities.

Can environmental RNA revolutionize biodiversity science?

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Molecular identification tools based on environmental DNA (eDNA) are expected to transform biodiversity science by enabling rapid conservation actions based on near real-time data of global coverage. Despite this promise, its broad adoption requires a drastic reduction in uncertainties due to false positives and negatives. These problems are often due to the persistence and transport of eDNA across large temporal and spatial scales. Thus, alternative markers with shorter half-time could potentially solve this problem. The use of environ-

mental RNA (eRNA) as a marker for species identification has received very little attention. This curious omission is likely due to the simple observation that, in laboratory conditions, RNA molecules are not stable, experiencing a 50-fold increased rate of degradation relative to DNA. Thus, RNA is expected to have ephemeral persistence outside the living organism, particularly in an aqueous environment. However, this assumption remains largely untested, and circumstantial evidence indicates the need for a close evaluation. I define eDNA eRNA as the complement of DNA or RNA, sampled directly from the environment, outside the progenitor organisms, and present in both cellular or extracellular form. I discuss the detectability of eRNA in aquatic and terrestrial settings by exploring broad research fields, spanning paleo-genetics, forensic science, cell biology, functional genomics, and molecular ecology. I suggest that eRNA could provide a more reliable signature of the community of metabolically active organisms present at a particular geographic or temporal location. Species identification tools based of eRNA are likely to support more accurate assessments of ecosystem structure and function. I emphasize the need for studying the mechanisms for RNA release, persistence, movement, and degradation in the environment.

Molecular identification of endangered marine predators by barcoding ancient museum rostra of Mediterranean sawfish populations (Chondrichthyes, Pristidae)

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Background: A growing concern in conserving threatened animals affected by human impact has been significant worldwide. Among marine animals, sawfishes (Chondrichthyes, Pristidae) are considered one of the most endangered families among elasmobranchs, resulting in extinction in many coastal areas around the world, including the Mediterranean Sea. Here, sawfish occurred with two species, *Pristis pristis* and *P. pectinata*, until the second half of the last century and are now considered Critically Endangered/Possibly Extinct. The historical occurrence of sawfish in the area is documented by bibliographic/archival records and by numerous preserved historical rostra available in museum collections. In this study we attempted to genetically characterize the historical remains of sawfish from several European museums and to enable the investigation of their evolutionary and ecological relationships with global samples. **Results:** A total of 80 rostra specimens, dated from 1700 to 1900 and catalogued as unknown origin or Mediterranean (11), were collected from 11 European museums and were properly prepared for ancient DNA genetic analysis. Taxonomic identification at the species level was obtained through PCR amplification of small fragments (~150 bp) of two mitochondrial markers commonly used for species identification (i.e., the mitochondrial COII and the NADH 2). Sequence comparison with currently available ones from public repositories and phylogenetic tree analyses indicated that the historical specimens belonged to four species, *P. pristis*, *P. zijsron*, *P. pectinata*, and *Anoxypristis cuspidate*, with a high frequency of mismatches (69%) between molecular identification and species museum cataloguing, when present. These preliminary data also showed the presence of two sequence sub-clusters in the poorly barcoded species *P. zijsron*. **Significance:** With the expansion of this initial analysis, we will contribute to increase the limited molecular data of these critically endangered large predators and to exploit historical genetic data for reconstructing phylogenetic/phylogeographic extent of the possibly extinct population of Mediterranean sawfish.

DNA barcoding Antigua and Barbuda

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Background: Due to climate change, pollution, invasive species, and other factors, species world-wide are vanishing at an alarming rate. This has important social and economic implications for Caribbean island nations. In addition, many students in the Caribbean, who may in the future become scientists involved in the conservation effort, are disadvantaged relative to other countries through a lack of science teaching infrastructure and (or) expertise. DNA barcoding is increasingly being introduced into biological science educational curricula, and such projects have been immensely valuable, both in educational terms and in assisting the worldwide conservation effort. This project aimed both to begin the process of cataloguing and mapping the flora on the island nation of Antigua and Barbuda for conservation purposes, and to provide molecular biology research skills to local Antiguan students and international undergraduate medical students. Thirty students took part in the project over 8 days, including 10 local Antiguan students from Antigua State College, and 20 undergraduate medical students from the American University of Antigua. **Results:** The project was highly successful, with students sampling over 100 local plant species and creating records for these on BOLD. Student feedback indicated a high degree of satisfaction with the project, survey comments suggesting students enjoyed being able to contribute to conservation work in their own country, and feeling they gained valuable molecular biology skills. Future directions include expanding the project to include more local Caribbean students and training local educators to introduce DNA barcoding projects into their courses. **Significance:** The project has used DNA barcoding for the first time to catalogue and map species on Antigua and has addressed significant inequities in terms of science educational opportunities for Caribbean students.

Successful field application of a novel universal method for rapid fish species identification

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Background: DNA barcoding has exposed the extent to which commercial fish species are mislabelled worldwide. This rampant phenomenon poses a risk in terms of consumers' health, economic loss, and hinders resource and conservation management. Despite the success of DNA barcoding in improving seafood traceability and enabling public awareness, leading to more stringent regulations in the European Union, there remains a need for simple, rapid, and portable DNA authentication tools. Rapid and cost-effective solutions for the authentication of specimens have been made possible by targeting segments of the mitochondrial COI barcode region with a set of fluorescent probes and by visualizing the melt curves using a portable real-time PCR instrument. In order to evaluate the portability and practicality of such a method, we tested the protocol aboard the R/V Cefas Endeavour during a 3-week survey in the southwest coast of England and the English Channel. **Results:** The pelagic ichthyofauna was collected using a mid-water trawl and allowed us to sample commercially important fish species. We successfully and rapidly identified most fish species sampled aboard the R/V Cefas Endeavour. We operated in rough weather, and devised a procedure that can become a reliable field tool for species authentication purposes, in both seafood market and wildlife forensic contexts. **Significance:** We successfully identified commercially important species using a fast and portable method in harsh field conditions without the need to sequence extracted DNA. Such methods could allow for the rapid and inexpensive identification of mislabelled or illegally traded species along the supply chain, without resorting to DNA sequencing.

Hidden fish biodiversity: using eDNA to characterise the hidden diversity of coastal fishes in South Africa

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Background: South Africa is the meeting point of the Atlantic and Indian Oceans, where marine biodiversity thrives and coastlines range from cool-temperate to tropical climates. This dynamic oceanographic regime supports over 2000 fishes that utilise a range of habitat types, including coastal seagrass meadows, mangrove forests, and rocky and sandy shores. Here, we describe the fish biodiversity of these near-shore habitats using a metabarcoding method that, when compared with traditional methods, provides rapid species lists with reduced bias and less reliance on taxonomic expertise. **Results:** We applied an aquatic environmental DNA (eDNA) metabarcoding approach for describing the distribution of South African coastal fishes. Extensive troubleshooting and method optimisation led to a biomonitoring workflow that encompassed multiple coastal habitat types and generated large-scale datasets with a seasonal component. We have established baseline knowledge for eDNA-based fish distribution in the region and are incorporating this data into marine spatial plans. **Significance:** We have demonstrated that eDNA metabarcoding is a useful biomonitoring tool for South African coastal fishes, proving successful across large spatial scales with the use of a single method that is inclusive of different coastal habitat types and climates, and across varying levels of coastal development and marine protection. Furthermore, we are enhancing the ongoing efforts of including genetic data into spatial planning, something that has proven beneficial to conservation outcomes, by using our eDNA datasets for creating priority maps that are accessible to policy makers.

DNA barcoding for conservation biogeography of nonbiting midges in South America: the case of *Polypedilum* (Chironomidae: Diptera)

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Background: There is a pressing need for effective biodiversity assessment tools to accelerate the pace at which biodiversity can be monitored and conserved in the face of anthropogenic perturbations. In this context, the South American realms comprise the most diverse biomes on earth in terms of both species richness and phylogenetic composition. However, when it comes to the nonbiting midge fauna (Diptera: Chironomidae), the knowledge of conservation remains fragmentary. The most difficult challenge in the Neotropics is that the region contains a significant proportion of unknown species belonging to polytypic genera. DNA barcoding is a promising tool for estimation of diversity, guaranteeing rapid and accurate assessment of the undescribed fauna. **Results:** We analysed the ability to delimit species in the megadiverse chironomid genus *Polypedilum* from 34 localities in South America using DNA barcodes. Our dataset consists of 184 DNA barcode sequences with a fragment length of at least 500 bp. A maximum likelihood tree based on this dataset comprises 78 well-separated clusters. Using Objective Clustering, the DNA barcodes cluster into 72–81 molecular operational taxonomic units (OTUs), depending on the threshold range (2%–7%). Our results show that most of the morphologically indistinguishable specimens represent separated species complexes at different localities, revealing hidden diversity relevant to the monitoring of aquatic ecosystems for protection and conservation. **Significance:** During the next 50 years, at least half of the species that inhabit the planet will disappear. Thus, it is clear that new conservation strategies must be developed for the evaluation of biodiversity. One of these should focus on biogeography. DNA data may provide an evolutionary framework to diversity estimates, incorporating genetic distance among species. Moreover, the use of molecular data permits biogeographers to create statistically testable hypotheses of dispersal and range expansion within the hypothetico-deductive framework without reference to formally described species.

Significant taxon sampling gaps in DNA databases from the best-studied marine habitat on Earth limit the operational use of metabarcoding

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Background: Metabarcoding of environmental DNA samples has been suggested to complement and ultimately replace traditional methods to assess biodiversity in aquatic environments using sieves and microscopes. Society currently spends significant resources on monitoring of ecosystems either directly, from government funding, or indirectly as a cost to business. The North Sea oil and gas industry is one example with ambitious environmental monitoring programs regularly sampling marine macrofauna on the seafloor surrounding the activities. In metabarcoding, a database of species genetic barcodes is necessary to match the sequence data and produce species lists. **Results:** We used a large species list from the North Sea and publicly available barcode databases to estimate the sampling gap remaining for marine macrofaunal barcodes. The North Sea probably represents one of the most-sampled marine areas and could therefore be considered as a "best case" for marine benthic metabarcoding work. Our study suggests that for the two most-used molecular markers, 18S and COI, the fraction of known North Sea taxa with publicly available species barcodes is 44% and 51%, respectively. To understand if rare species are over represented in the taxa remaining to barcode, we calculated the fraction of the "top-ten" species in the database, resulting in a small increase in available barcodes only. **Significance:** We conclude that compared to global figures this area is relatively well sampled for DNA barcodes, but that a significant effort remains to fill barcode databases to levels that would make metabarcoding operational as a taxonomic tool.

Overlooked biodiversity: Fungi in Norway's coastal heathlands

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Background: Coastal heathlands are semi-natural, predominantly treeless landscapes created by low-intensity farming including grazing and prescribed burning regimes. This nature type is threatened in much of Europe, with land-use change, urbanization, and nitrogen deposition all contributing to further loss of these landscapes. Heathlands are characterized by poor, shallow soils and vegetation dominated by ericaceous shrubs. The Fungi in Coastal Heathlands (FiNCH) project represents the first attempt to comprehensively inventory and begin mapping the distribution of ascomycetous fungi found both above and below ground in Norway's coastal heathlands. **Results:** To date, more than 20 fungal species have been cultured and identified from the roots of *Calluna vulgaris*, *Vaccinium* spp., and *Erica* spp. growing in coastal heathland habitats. The aboveground ascomycete mycoflora consists primarily of inconspicuous species, and includes both saprotrophs and pathogens of the shrubs and bryophytes that are the dominant vegetation components. Fruitbody surveys and moist chamber incubations have identified a number of species, including records of species not previously detected in Norway, such as *Godronia andromedae*

and *Physalospora vitis-idaeae*. **Significance:** The FiNCH project indicates that despite hosting relatively low plant biodiversity, coastal heathlands are home to a broad diversity of fungi that have otherwise been overlooked in the Norwegian mycoflora.

Savanna tree origins and the paleoenvironment of our hominin ancestors

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Background: The savanna hypothesis, though controversial, continues to motivate research into the palaeo-environments of Africa. Reconstruction of these ancient environments has depended heavily on carbon isotopic analysis of fossil bones and palaeosols. The limited number of information in the fossil record, however, imposes a hamper on the strength of inference that can be drawn from such data. Time-calibrated phylogenies offer an additional tool for dating the spread of savanna habitat, especially in regions where fossils are rare or absent. Here, we generated a calibrated phylogenetic tree, using the core DNA barcoding regions rbcLa and matK of ~1800 woody taxa of sub-Saharan Africa. We then inferred the age of African savanna by examining the evolutionary splits between tree species that likely diverged in the savanna biome. **Results:** We show that the expansion of savanna occurred along a latitudinal gradient, appearing first in the tropics ~10 Mya, and then extending to southern latitudes over the next several millions of years, reaching southern Africa ~3 Mya. Furthermore, our results are consistent with the savanna hypothesis of early hominin evolution, and reignite the debate on the drivers of savanna expansion. **Significance:** Our analysis demonstrates the utility of phylogenetic proxies for dating major ecological transitions in geological time, especially in regions where fossils are rare or absent or occur in discontinuous sediments. Thus, we advocate the use of phylogenetic data as a valuable source of information on past environments in Africa.

Metabarcoding in herbal product authentication — where are we today?

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Background: Herbal products and other over-the-counter (OTC) drugs have limited medical oversight, frequent off-label use, and insufficient monitoring of adverse drug reactions. Many herbal products have a long history of use, but there are rising concerns over product efficacy, safety, and quality in the wake of recent cases exposing discrepancies between labelling and constituents. Quality and authentication assessment methods rely on morphology and analytical phytochemistry-based methods detailed in pharmacopoeias, but a variety of innovative methods have emerged. Herbal products, however, are often highly processed with numerous ingredients, and even if these analytical methods are accurate for quality control of specific lead or marker compounds, they are of limited suitability for the authentication of biological ingredients. Amplicon DNA metabarcoding is a high-throughput sequencing based method for molecular identification using DNA barcoding that has been shown to enable accurate species identification for products that contain mixtures of DNA from different species. **Results:** Different methods of quality control and authentication have varying resolution and usefulness along the value chain of these products. DNA barcoding can be used for authenticating products based on single herbal ingredients and DNA metabarcoding for assessment of species diversity in processed products. **Significance:** DNA barcoding and metabarcoding have potential in the context of quality control of both well and poorly regulated supply systems. Innovations in DNA-based identification will further refine these approaches, and help consolidate the niche in which these methods augment current authentication.

GO-SEE: Global Ocean Systems Ecology & Evolution

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In between the small (atoms, cells, and human body) and the large (weather, stars, and the universe), where trillions of research dollars have been spent, there are "eco-systems"—self-organized and evolving networks of life interacting with physico-chemical processes at micro-to-planetary scales. Ecosystems have shaped our atmosphere and biogeochemical cycles, they currently buffer climate change and provide food and medicine, and their health will determine the future habitability of our planet. Due to their extreme complexity, mixing biology, chemistry, and physics, ecosystems have long escaped holistic quantitative assessment. In 2009, a small interdisciplinary team assembled around the schooner Tara with the goal to quantify and model a primary complex oxygenated ecosystem on Earth: that of plankton. Through two circum-navigations of the world's ocean between 2009 and 2014, we systematically sampled entire plankton ecosystems down to 1000 m, from polar to tropical waters and from viruses to small animals, generating the largest collection of homogeneous samples and eco-morpho-genetic data from any single biome. Amongst others, the Tara Oceans data have shown that (*i*) although plankton diversity (genes, species, functions) is much higher and unknown than previously recognized, it is measurable with high-throughput sequencing and imaging technologies, including (meta)barcoding; (*ii*) along the entire life taxonomic/size spectrum, biodiversity peaks in unicellular eukaryotes (protists) and not in viruses, bacteria, or animals, a pattern most likely driven primarily by organismal complexification through symbiogenesis; and (*iii*) ocean circulation and physico-chemical parameters explain a minor part of plankton community composition; all kind of biotic interactions (predation, parasitism, commensalism, mutualism) and top-down ecological processes appear to be the primary drivers of macro-ecological patterns (e.g., global interactome, biogeography, virus as driver of carbon pump, etc.). Altogether, today we see plankton as a planetary, dynamical and multi-layered network that acclimates and adapts to local environmental conditions, essentially through multiple modes of life-driven interactions (red-ox meta-metabolomes, chemical signaling via metabolites and vesicles, organismal symbioses sensu lato). Due to its holistic tractability, relatively fast eco-evolutionary dynamics, and continuous fossil record, the global marine plankton interactome offers one of the best natural laboratories to understand the mechanisms of life and complexification, and to develop in the next century a robust quantitative theory of ecosystems dynamics and patterning.

Using pollen DNA metabarcoding to investigate floral visitation by honeybees and wild pollinators

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Background: Pollination is a vital ecosystem service and a key consideration for food security. Despite their importance, pollinators are

facing declines throughout the world due to habitat loss, agricultural intensification, pests, disease, and climate change. Pollinating insects require access to suitable plants for foraging, and as native habitats decrease gardens may become increasingly important refuges. We use pollen DNA metabarcoding to investigate which plants different pollinator groups visit throughout the year and the extent to which these can be provided within gardens. **Results:** The National Botanic Garden of Wales and adjacent organic farmland have been used to assess plant use by different pollinator groups (bumblebees, solitary bees, honeybee, and hoverflies) in order to build a temporal and spatial picture of foraging. Each month, from April to September, all plants in flower throughout the study site were recorded, honey was sampled from six hives (from two apiaries), and wild pollinators were collected from a series of transects. We have used DNA metabarcoding to survey which plants pollinators use by assessing the pollen biodiversity within honey and from the bodies of insects, using rbcL and ITS2. To complement this work, we have also analysed 475 honey samples provided by beekeepers throughout the UK to survey which plants honeybees use on a wider scale. Initial results show that only a small proportion of available flowering plants are visited by honeybees within a diverse landscape. The greatest proportion of DNA comes from a small number of native plants, including *Rubus fruticosus*, *Trifolium repens*, and *Taraxacum officinale*, supplemented with lower levels of horticultural species. **Significance:** We are using our findings to develop evidence-based guidance on plants for pollinators and are working with specialist plant nurseries to pilot a "Plants for Pollinators Assurance Scheme".

Counting with DNA in environmental metabarcoding studies: are sequence counts useful?

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Many biodiversity studies make use of metabarcoding, an approach which combines high-throughput sequencing (HTS) with DNA barcoding to characterise organisms in complex mixtures (e.g., in eDNA or bulk samples). The approach is also used to study animal diet by analysing food DNA in faecal samples. One feature of HTS is that it provides counts of DNA sequences and therefore has the potential to provide not only a qualitative list, but also a quantitative assessment of what DNA is present. But what do these counts mean, and do they reflect biomass of metazoans in a sample? Is it reasonable to use read proportions to retrieve semi-quantitative information, or should we work strictly with presence/absence datasets? Here, we explore how sequence counts are used in metabarcoding studies and discuss results from our research on animal diet (seals and seabirds) and zooplankton communities. We point out that summaries based on frequency of occurrence data have their own biases and argue that in some situations the quantitative interpretation of count data can be justified. We also outline our use of correction factors to account for taxa-specific recovery biases and the inclusion of internal standards to enable quantitative comparisons between samples.

Global measure of the terrestrial biosphere by understanding biogeochemical cycling of environmental DNA

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The biosphere is under immense pressure, and the Intergovernmental Panel on Biodiversity and Ecosystem Services (IPBES) goal is to "perform regular and timely assessments of knowledge on biodiversity … at the global level". It is established that we need standard measures of Essential Biodiversity Variables (EBVs) to execute regular and timely assessments of knowledge on global biodiversity, but many conventional methods will not be feasible. Thus, there is great need to establish new ways to measure biodiversity because current methods are too expensive, time intensive, and hard to do for multiple species and on large geographic regions. Environmental DNA (eDNA) has the potential to solve this bottleneck. eDNA is DNA shed from an animal or plant into its surroundings that can be collected and sequenced to infer species presence. Precise knowledge on the fate of eDNA is crucial to facilitate the paradigm shift to a different, powerful way to assess biodiversity, enable the interpretation of results in a broader geographic scale, and tap the full potential of the technology. The advantages of using eDNA are exciting; however, using a complex molecule in the environment, rather than direct observation of an individual, creates important risks. As we are still exploring how eDNA from currently living animals and plants persists in space and time, I will discuss the need to establish, and evidence for, a general model for eDNA decay in the environment. I will highlight how biodiversity information in the form of transported eDNA from both aquatic and terrestrial organisms can be harnessed and present my vision for how to sample eDNA from naturally occurring biodiversity accumulators to vastly change the cost, speed, and geographic scale with which to survey biodiversity to empower the future of real-time biodiversity monitoring on a global scale.

The fidelity of DNA barcodes from museum specimens: no cause for concern

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Background: The recovery of DNA barcodes from museum specimens poses some difficulty as their tissues often contain low concentrations of degraded DNA. Post-mortem DNA damage has been examined for fossil and subfossil samples, but little effort has been directed toward analyzing patterns of DNA degradation in museum specimens. The large amount of data generated by HTS makes it possible to detect rare misincorporation events, which would be overlooked when only a few clones of PCR products are investigated. **Results:** Substitution patterns were compared for five old (average age = 71 years) and five recently collected butterflies to ascertain the error rates introduced by Platinum Taq polymerase from nucleotide misincorporations induced by damage to the COI barcode region. Museum and fresh specimens displayed highly significant differences for the transition C/G > T/A ($p < 0.001$). The comparison also revealed weaker but significant differences for the transversions A/T > T/A ($p = 0.003$), C/G > A/T ($p = 0.02$), and T/A > G/C ($p = 0.03$). Overall, error rates induced by DNA degradation were relatively low—the most common change (C/G > T/A) occurred at the frequency of about 1 in a 1000 bp, while the frequency of the other substitutions was less than a tenth as high. **Significance:** This case study has employed the COI gene in butterflies as a model system to examine the frequency of nucleotide misincorporations in sequences recovered from museum samples. The overall results are reassuring—the incidence of nucleotide substitutions induced by damage is so low that it will not impede the recovery of valid barcode records from the oldest museum specimens.

The quest for the Pole: are larvae of boreal benthic species already capable of reaching the Arctic Ocean?

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Background: Waters advected into the Arctic from sub-Arctic seas carry nutrients and living organisms. Many marine benthic invertebrates release planktonic larvae (meroplankton) which can drift with

water currents. Because of their sometimes months-long residence time in the water column, some planktonic larvae have the potential to disperse over long distances before settling to the seafloor. This implies that sub-Arctic (boreal) species can potentially reach the Arctic during their larval stages, though not all as yet find suitable conditions to settle and survive to adulthood. The goal of this study is to determine the community composition of benthic invertebrates at the larval stage and which sub-Arctic species are currently entering the Arctic at these early stages. **Results:** Meroplankton was collected on the northern Barents Sea shelf, Arctic Ocean, in November 2017, as well as in January, April, July, and August 2018. This unprecedented seasonal sampling in open waters of the Arctic provides crucial coverage of the species present in the larval pool, and permits resolution of seasonal occurrence that has never been available before. Individual larvae were assigned to species using a combination of morphological analysis and DNA barcoding using the COI Leray-XT primer set. Our novel approach to DNA barcoding has yielded much higher identification success rates (up to 92%) than previous studies. Preliminary analysis suggests that larvae are present throughout the year and that echinoderm, bivalve, and polychaete larvae dominate the larval community. **Significance:** This knowledge can help predict which boreal species are likely to invade the Arctic as the climate continues to warm, and can offer a point of reference for future studies.

Barcoding marine fouling crustaceans from Brazil: new insights into bioinvasion and cryptic diversity

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Background: In marine environments, submerged artificial substrates are usually rapidly colonized by fouling communities. Sessile alien species such as ascidians, bivalves, barnacles, and polychaetes are comparably well studied on a global scale, while the mobile portion of these communities is usually underestimated or largely ignored. Constituting up to 80% of mobile fouling species, crustaceans represent the most diverse and abundant taxon. Despite their high species richness, crustacean fouling assemblages across the globe usually display high similarity among them, often harbouring congeneric species or even the same cosmopolitan species. The present project aims to fill knowledge gaps on the connectivity between fouling communities, elucidating possible pathways for marine bioinvasions. Here, we initiated a crustacean barcode library for fouling communities in Brazilian subtropical bays and harbours, along the coasts of southern São Paulo to the Santa Catarina states. **Results:** Twenty-eight molecular operational taxonomic units (MOTUs) were delineated among 22 morphologically identified species. Around 35% of the specimens had no match in neither BOLD nor GenBank (identity lower than 90%), leaving more than 50% of the MOTUs without taxonomic identity. Moreover, four species matched 100% with amphipod species from the Atlantic coast of Florida. This corroborates a marine Invasion Risk indicating that North American Atlantic coasts may be an invasive species donor for Brazilian systems. Interestingly, three of these species had multiple clusters that were separated by comparably large genetic distances, indicating the presence of cryptic species with populations in Brazilian, European, and North American waters. **Significance:** Our results represent a start for assessing community composition and connectivity of Brazilian fouling crustaceans. Furthermore, they call for better knowledge of species identities and underline how comprehensive DNA-barcode libraries of fouling species might be a valuable tool to identify invasion routes among geographically different marine realms.

Agricultural bio-surveillance via metabarcoding of bulk Malaise trap samples

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Background: The wedding of mass-trapping and high-throughput sequencing is enabling the establishment of bio-surveillance programs that deliver near real-time information on resident biodiversity and its variation over time and space. The agricultural sector stands to benefit greatly from the bio-monitoring of crops and surrounding areas, not only to optimize the application of pesticides and biological control agents, but also to guide the management of adjacent properties to maximize yields and enhance local biodiversity. We report the development of an agricultural monitoring protocol based on metabarcoding of bulk Malaise trap samples. The protocol was employed across a wide gradient of land use, from intense corn–soybean farms, to ALUS (Alternative Land Use Services) farms with varying restoration of adjacent land, to natural protected areas. **Results:** From mid-April to mid-October 2018, 64 standard Malaise traps were deployed on 32 ALUS properties, conventional farms, and conservation areas in Ontario, Canada. The 683 two-week trap samples underwent lysis, DNA extraction, and COI amplification in bulk, and the 462 base pair amplicons were then pooled prior to sequencing on an Ion Torrent S5 system. Data processing and taxonomic assignment were executed using mBRAVE, followed by a new denoising routine. Approximately 1.6M arthropods were analyzed, resulting in 262K BIN (barcode index number) occurrence records, representing 18K unique BINs from 40 orders and 550 families. BIN richness varied considerably between individual samples (range = 175–562, mean = 386 BINs per sample) and across the gradient of land use (range = 1142–2966, mean = 2086 BINs per site). **Significance:** This work has generated a rich dataset to explore the full suite of factors influencing arthropod biodiversity in relation to farming practices and landscape heterogeneity. It also validates a standardized methodology for rapid biodiversity surveillance in agricultural settings, and establishes a framework for tracking pests, control agents, and beneficial taxa.

The conundrum of species delimitation: a genomic perspective on a mitogenetically super-variable butterfly

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Background: The increasing use of molecular data in the study of global biodiversity is revealing unexpectedly high levels of genetic differentiation in numerous species across all groups of organisms. Most such cases are based on mitochondrial DNA (mtDNA), mostly fuelled by a rapidly increasing number of DNA barcodes. However, the interpretation of such patterns is subject to debate and largely caused by a lack of comprehensive genomic data used to assess deep intraspecific divergence in mtDNA. The butterfly *Melitaea didyma* (Lepidoptera, Nymphalidae) stands out as one of the most striking cases of intraspecific genetic differentiation detected in Palearctic Lepidoptera: 11 partially sympatric mitochondrial lineages have been reported, displaying levels of divergence of up to 7.4%. **Results:** To better understand the evolutionary meaning of such mtDNA lineages, we compared mtDNA and genome-wide data using double-digest restriction site-associated DNA sequencing (ddRADseq) from 93 specimens of *M. didyma* ranging from Morocco to eastern Kazakhstan. *Wolbachia*

screening was also performed on all specimens. We found only a partial match between ddRADseq and mtDNA results since some mtDNA lineages have likely resulted from introgression events and were potentially affected by *Wolbachia* infection. The five ddRADseq clades detected were allopatric, displayed high pairwise FST values, and were assigned to species by Bayes factor delimitation analyses. Our analyses also estimated various levels of gene flow between some of the clades. **Significance:** *Melitaea didyma* represents the first case of deep mtDNA splits among European butterflies assessed by a genome-wide DNA analysis and shows that interpretation of patterns remains complex even when a high amount of genomic data is available. These findings highlight the need for a consensus on species delimitation in allopatry, an issue of relevance to a significant proportion of global biodiversity.

The Kruger Malaise Program: understanding the role national parks play in supporting biodiversity

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Background: The major decrease in insect biomass observed at sites in the northern hemisphere over the past 30 years may be linked to the intensification of agricultural activity and associated pesticide/herbicide usage. What is happening to insect diversity and biomass in other regions that have been less impacted by agriculture? With an area of 19 500 km^2, Kruger National Park (KNP) is the largest of South Africa's 19 national parks. Understanding the insect community in KNP could play a crucial role in deciphering the link between decreases in insect biomass and commercial agriculture. **Results:** Launched in May 2018, the Kruger Malaise Program (KMP) is examining spatial and temporal patterns of variation in the species diversity and biomass of arthropod communities in KNP by coupling a year-long sampling program with DNA barcode analysis of the resultant specimens. Twenty-six Malaise traps have been deployed in all 22 sections of the park, providing coverage for 11 of the 15 vegetation types present. Trap catches are harvested weekly by rangers and dispatched quarterly to the Centre for Biodiversity Genomics in Guelph, Canada, for biomass determination and sequence analysis. By November 2018, 590 weekly samples had been collected, and half these samples are slated for single-specimen analysis. Current results, based on the analysis of more than 100 000 specimens, indicate high diversity and marked spatial and seasonal shifts in species composition. **Significance:** By combining advanced sequencing technology with simple sampling protocols, this project is enabling knowledge sharing between conservation managers and scientists. The KMP will help to show how work in national parks can help to fast-track biodiversity assessment on a global scale.

Description of a new *Nitzschia* species from saline bomb crater ponds

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Background: During World War II, mistargeted bombing created craters in a moderately saline wetland area of the plain of Danube-Tisza Interfluve, Hungary. Astatic saline ponds formed in the bomb craters provide special and vulnerable habitat for a unique biota. We investigated epiphytic diatom communities of these water bodies, evaluating their ecological status. Water quality assessment requires accurate identification of species. One of the dominant species could not be identified as any previously described species. Therefore, we subjected field samples and a pure culture established from isolated cells to detailed light and scanning electron microscopy investigation and phylogenetic analysis of multiple genes (18S and 28S rDNA, rbcL). **Results:** Based on morphological features, the unknown taxon mostly resembled *Nitzschia frustulum*. However, in contrast to *N. frustulum*, it did not have central nodule and had fewer fibulae in 10 μm. Phylogenetic analyses showed nonmonophyly of the genus *Nitzschia*, similar to previous studies. In accordance with the results of our morphological study, the molecular investigation revealed a close relationship of the investigated taxon with *N. frustulum* and related taxa (e.g., *N. inconspicua*, *N. supralitorea*), but it clearly separated from them. **Significance:** Overall, our study suggested that the studied taxon is a species of the genus *Nitzschia* that has not been described before. We therefore proposed the new name *Nitzschia reskoi* Ács, Duleba, C.E.Wetzel & Ector for this taxon. Ecological investigation indicated that increasing abundance of *N. reskoi* was a signal of the degradation of the intermittent saline wetlands.

Five years as national research infrastructure: status of the Norwegian Barcode of Life Network (NorBOL)

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Background: The Norwegian Barcode of Life (NorBOL, www.norbol.org) is both a national network and national research infrastructure for DNA barcoding in Norway. NorBOL has existed for 12 years as a network, with increased activity in the last five years enabled by grants from the Research Council of Norway and the Norwegian Biodiversity Information Centre. The main goals of the grant period has been to barcode 20 000 species from Norway and Polar regions, and at the same time build a distributed network of competence and expertise in DNA barcoding. NorBOL is a member of iBOL, collaborates closely with the Centre for Biodiversity Genomics at the University of Guelph, and uses BOLD as repository of animal, plant, and fungal barcodes. **Results:** Currently, there are more than 96 500 specimen records of 22 500 species originating from Norway or NorBOL in BOLD. The records are represented by 49 616 formal barcodes of 16 248 species and 13 213 BINs. In addition, there are sequences from about 2400 species associated with Norwegian specimens in BOLD that do not have formal barcode status. Thus, about 18 600 species from Norway have sequence information in BOLD. This constitutes about 31% of the estimated species diversity in the country. There are about 270 researchers and students involved in DNA barcoding in Norway, with most of the activity centered around the universities and the larger research institutes. Currently, 250 entries (publications and other activities) are linked to NorBOL in the National Research Information System CRIStin. **Significance:** The establishment of NorBOL as a distributed national research infrastructure on DNA barcoding has enabled a number of applied projects linked to biodiversity research and management. In concert with other initiatives in Europe, NorBOL has contributed significantly to the barcode library of multicellular life in northern Europe.

The ultimate primer comparison for metabarcoding terrestrial insects and aquatic invertebrates

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Background: DNA metabarcoding is an excellent tool for rapid assessment of biodiversity, but it requires proper protocols and in particular appropriate primer sets. The lack thereof will result in substantial bias, and will compromise taxon detection. Thus, we set out to thoroughly evaluate commonly used primer sets, as well as newly developed ones. We did metabarcoding of two mock communities each containing hundreds of taxa, using 21 different primer sets. The terrestrial community contained flying insects, while the aquatic community comprised of benthic macroinvertebrates. **Results:** Our results show several primer sets which each recover over 95% of the species in both communities, while some primers show rather poor performance. Our data also clearly show that setting off primer bias by using several primer sets does not improve recovery. A single primer set appropriately designed and validated for groups in question is sufficient. **Significance:** Designing metabarcoding primer sets is a difficult and challenging task, but skipping primer development and evaluation in favour of primers that lack degeneracy or by multiple marker approaches, cannot be the answer. We clearly show that there are plenty of well-designed degenerate primer sets suitable for insect metabarcoding. They just need to be used.

A case of direct application of DNA barcodes in medical diagnosis: Lagochilascarosis, a rare human disease in northern Neotropics

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Background: The occurrence of a rare nematode parasite, *Lagochilascaris minor*, from a human host in the tropics of Mexico is presented herein. It usually is found in wild cats, such as jaguars or pumas, but it can also parasitize domestic cats and dogs. This nematode has been reported in the Americas, although it is much more common in South America, where the eggs have been found in public parks. However, the identification of the latter with common methods can be misleading due to their resemblance with other ascaridoid eggs. **Results:** The parasite caused destruction of the mastoid apophysis and lateral sinus, and the cerebellum was compromised in a 23-year-old patient. After a radical mastoidectomy and a treatment with 200 mg oral albendazol during 63 days, we observed a total recovery of the person. *Lagochilascaris minor* was identified with DNA barcodes and morphology. It is unknown how infection occurred in this case, although it could be after direct exposure to eggs or consumption of uncooked wild meat. High-quality sequences of the COI gene (DNA barcodes) were obtained after using semi-degenerate primers designed for microcrustaceans. A comparison with 81 ascaridoids obtained from Barcode of Life Data Systems (BOLD) shows a unique clade for it, with the closest relative being *Baylisascaris procyonis*. The diagnosis by using DNA barcoding will allow the recognition of the infection parameters, transmission, and more precise epidemiology of this parasite. **Significance:** To our knowledge, this is the first multidisciplinary study involving DNA barcodes as a diagnostic medical tool in a human patient. This field of research can be promising because we can get a precise identification of the parasites in any stage of their life cycle. With this information, we can not only diagnose the disease, but also prevent it, by finding the infective stages in the environment, the intermediate hosts, or the vectors.

Relevance of DNA barcode baselines in tropical freshwater conservation and species discovery

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Background: Recently, development and popularization of new technologies in DNA sequencing allows researchers to obtain vast amounts of sequences in any environment. Nevertheless, most of these sequences yield only an approximation of the species to which they belong, but not a real species identity. This problem is significant in freshwater ecosystems, where many species are still unknown to science. **Results:** Mexico, we believe, is one of the most advanced countries in terms of having a barcode reference library of freshwater species, including 137 species of rotifers, 183 cladocerans, and 104 copepods. In addition, many other taxa not traditionally considered as zooplankton, e.g., chironomids, acarii, ostracods, and other meroplanktonic larvae, are represented. These data can be used together with new sequencing technologies to monitor changes in the planktonic communities, not only as a result of pollution, but also to detect introductions of exotic species. For example, we found the planktonic larvae of *Mytilopsis sallei*, considered invasive in Bacalar Lake (Mexico), to be conspecific with specimens from Lake Izabal (Guatemala) (type locality), and different from the invasive form found in Southeast Asia and Australia. We also present the first results from our studies in the Colombian Amazon basin, where many overlooked species, mainly within the Argulidae, Chydoridae, and Scapholeberinae, re-appeared after more than 20 years of surveys with traditional methods. **Significance:** Next-generation sequencing techniques together with baseline DNA barcode reference libraries will become a powerful tool for freshwater conservation worldwide. We regard these types of analyses are much more valuable that traditional approaches because they are based on the whole communities and not only a few flagship species. By analyzing full communities, we can get a more holistic view of what is happening in a freshwater ecosystem.

Unveiling the hidden diversity of soil-dwelling oribatid mites (Acari: Oribatida) of Finland with DNA barcoding

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Background: A substantial portion of soil mesofauna consists of oribatid mites, which are microscopic (0.1–1 mm) soil-dwelling decomposers. In boreal and temperate forests, their abundance may yield up to 200 000 specimens and 50 species/m^2 of soil. Their morphological identification is a challenge, for which DNA barcoding may provide a practical tool. Unfortunately, oribatid sequence data are still largely lacking in reference databases. This study presents the progress towards the first taxonomically comprehensive DNA barcode reference library for oribatid mites using the fauna of Finland, where about 350 species have been recorded. **Results:** Originally, a total of 984 specimens representing 163 named species were investigated using the COI barcode region. Out of these, 450 sequences were obtained (45% success rate) varying between 157 and 658 bp. In total, 235 sequences were barcode compliant, but all sequences were included in the taxonomic analysis. The sequences represented 123 named species belonging to 80 genera and 45 families. The maximum likelihood tree supported 80% of the named species (101 species; but 25 species were singletons or doubletons). Interestingly, 22 named species were split into two to five haplotypes, suggesting cryptic species. Altogether, the barcodes represented 152 haplotypes. The interspecific p-distances were 9.1%–45.2% (average 27.6%), and intraspecific p-distances 0.0%–7.1% (average 0.8%). Haplotype sharing between named species was rare, and those cases were considered misidentification or contamination. **Significance:** The results confirm that DNA barcodes are an effective tool for the iden-

tification of oribatids. The haplotype diversity was 25% higher than the identified species, suggesting the presence of hidden diversity. This study provided barcodes for over 120 oribatid species, of which 74 were novel barcodes of species according to GenBank. Until now, 30% of the Finnish oribatid fauna has been barcoded, and the project continues with efforts in increasing sequencing success and wider taxon sampling.

Drivers of diversity and future scenarios for Arctic ecosystems from ethno-ecology, contemporary ecology, and ancient environmental DNA

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The Arctic is currently experiencing dramatic ecosystem changes, with immediate effects on ecosystem services connected to food production, climate regulation, natural resources, and cultural integrity. Arctic and Subarctic communities, whose livelihoods are closely linked to their environment and who directly depend on the herding and hunting of large herbivores, will have to adapt to the effects of climate warming and vegetation changes. Building relevant scenarios thus requires understanding the relative roles of climate, herbivory, and increased anthropogenic pressures as large-scale drivers as well as on local scales relevant to the communities. This has, however, been precluded by the scarcity of long-term (millennial) data sets elucidating climate and land use changes. Palaeorecords offer a unique possibility to fill this gap. In the BiodivERsA project "Future ArcTic Ecosystems (FATE): drivers of diversity and future scenarios from ethno-ecology, contemporary ecology and ancient DNA" we are conducting a comprehensive inter- and transdisciplinary study using ancient environmental DNA from sediment cores, current ecological observations, and anthropological investigations of indigenous and local knowledge and interpretations. To reconstruct past vegetation changes over large spatial (circumarctic) and temporal (Last Glacial Maximum until today) scales, and investigate to what extent changes are driven by herbivory or by climate, we will analyse sediment core DNA for plants, mammals, and fungi. This will generate datasets with a high taxonomic resolution, allowing us to make detailed inferences about questions of palatability and potential toxicity of plant to herbivores, as well as refine reconstructions of past ecological conditions. These data will be compared to and integrated with data from modern ecological experiments, as well as with indigenous and local knowledge and interpretations. By coupling these datasets and evaluating their respective and joint explanatory power, we will create scenarios that support decision-making in the face of accelerating socio-ecological transformations throughout the circumpolar North.

DNA barcode libraries of clitellate worms (Annelida): challenges and possibilities

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Background: Soft-bodied invertebrate taxonomy is often cumbersome, due to prevalence of cryptic speciation, sometimes in combination with great intraspecific genetic variation. Molecular systematics alleviates this situation, but species delimitation using a single DNA barcode is not trivial. Instead, a multilocus approach, involving mitochondrial as well as nuclear markers, and large samples of specimens/populations are required. Morphological data should be considered too. A species cannot be securely identified using a single locus, until its taxonomy has been properly resolved, and then it may need to be represented by a range of different haplotypes in reference libraries. **Results:** These challenges are evident in a long-term survey of worms belonging to the annelid class Clitellata in Scandinavia. To date, this work has resulted in COI barcodes of 16 000 specimens. Initial COI analyses overestimated species diversity, and integration of nuclear markers (ITS, H3) from the same material showed that there is no standard COI-barcoding gap in these animals. Multilocus species delimitation analyses suggest that they represent >500 different species, but intra- and interspecific COI-distances overlap greatly; each species must be investigated individually. This endeavor now enters its final phase, i.e., to establish nearly complete DNA-barcode libraries of the Scandinavian clitellates. Nearly complete refers to (as far as possible) the coverage of species, and haplotype diversity within species. As COI may be unsuitable for applications involving environmental, ancient, or otherwise degraded DNA, a second mitochondrial barcode, 16S, will be uploaded on public databases too. Finally, the project will generate libraries for sequences of the ITS region and H3. **Significance:** The vision is that COI and 16S libraries will give reliable matches (99%–100% similarity) with a vast majority of clitellate specimens in Scandinavia. The more complete the libraries become, also with species from elsewhere, the easier it will be to discover previously unnoticed taxa.

The COI gene as an indicator of mitochondrial adaptation to hypoxic high-altitude environments in birds

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Background: Birds that inhabit highland environments tolerate hypoxia due to physiological and morphological adaptations. Even though mitochondrial genes participate in the cellular respiratory process, their role in the adaptation to hypoxia has been poorly studied, and analyses are needed to establish general patterns of mitochondrial adaptation to high altitude. In this context, we studied the adaptation of COI to hypoxia. The choice of this gene is based on two main reasons: (*i*) it catalyzes the last reduction of oxygen in the electron transport chain, and (*ii*) large-scale sequence libraries of a portion of this gene are available due to the Barcode of Life project. Over 22 000 sequences of the COI barcode region were retrieved from around 2000 avian species from the American Continent. We classified 155 pairs of sister species into highland–lowland, highland–highland, and lowland–lowland species pairs to compare their COI barcode region sequences. Also, because the COI barcode region represents only a portion of this gene, we sequenced the complete gene for a reduced set of 28 sister species pairs. **Results:** Analyses based on the COI barcode region pointed towards more changes in amino acids when at least one of the species of the pair was a highland species. However, results did not show a clear trend towards more significant modifications or a stronger effect on the protein in high-altitude species. Results were more consistent with the smaller dataset of complete protein sequences, showing more changes between species when at least one of them inhabited highland environments and also indicating that changes were more significant for the protein function in highland species. **Significance:** This is the first large-scale analysis of mitochondrial differentiation in high-altitude species in any taxonomic group, and it suggests that the mitochondrial genome is involved in the adaptation to high, hypoxic environments in birds.

Working towards standards in environmental DNA biomonitoring of marine ecosystems

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Background: The Centre for Environmental Genomics Applications (CEGA) is an independent research facility in eastern Canada dedicated to the development of environmental DNA (eDNA) approaches to biomonitoring with a focus on marine environments. By leveraging a multidisciplinary team of experts and the latest sequencing technologies, CEGA aims to create a path that brings us from small-scale pilot projects to widespread regulatory and industry acceptance of eDNA-based assessments. **Results:** We present the results of a single eDNA sampling mission in Conception Bay, Newfoundland, Canada. We explore the optimization of biodiversity recovery in this coastal marine ecosystem through ultra-deep sequencing (Illumina NovaSeq) and sampling design (collection volume and sampling replicates). Using only general eukaryotic primer sets, this high-quality, deep sequencing enabled biodiversity analysis of rare, endangered, and invasive species in addition to the dominant taxa. **Significance:** Our efforts to create standards in this field benefit from close relationships with industry partners, academic institutions, and regulator collaborations, which in turn will facilitate adoption of these techniques.

Small-scale spatial variation of meiofaunal communities in Rio Lima estuary (NW Portugal) assessed through metabarcoding

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Background: DNA metabarcoding provides a practical approach to investigate communities hard to track through traditional morphological approaches as, for instance, the estuarine meiofauna. In this study, we examined the patterns of small-scale spatial variation of meiofaunal communities along a salinity gradient in the Rio Lima estuary, NW Portugal. We collected sediments during low tide at four sampling stations along the salinity gradient (range 9.10–28.31) and, in each station, six sampling points were used: three in the high intertidal and three in the mid-intertidal zone. Within each zone, sampling points were ~4–5 m apart. The meiofauna metagenome was isolated directly from the sediment (10 g), and submitted to PCR amplification targeting a sub-region of the mitochondrial cytochrome oxidase I and the V4 region of the 18S rRNA genes. Sequencing was carried out in a MiSeq platform and resultant reads submitted to a customized pipeline for size and quality filtering before clustering into operational taxonomic units (OTUs). Representative reads of each OTU were BLASTed against the nt GenBank database and taxonomically assigned by MEGAN. **Results:** The number of meiofauna OTUs reached saturation at six sampling points in all stations. However, the spatial structure of these communities was quite high, with an average OTU overlap within and between intertidal levels lower than 50% and within each site for both targeted genetic regions. Moreover, the OTU overlap among the four sampling sites ranged between ~13%–30% (COI) and 20%–31% (V4). **Significance:** Both targeted genetic regions indicated high spatial variation in the meiofauna, suggesting considerable patchiness in these communities within Lima estuary, apparently superior to that reported in the literature for other estuaries. This high variation within stations blurred the discrimination of the communities along the salinity gradient. Hence, the sampling design requires careful consideration for future metabarcoding-based meiofaunal surveys.

Popular seafood labels threaten biodiversity of cação (shark), merluza (hake), and linguado (flatfish) sold on the Brazilian market

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Background: The collapse of many commercially important fish stocks has led to an intense focus on seafood identification to support consumer-driven sustainable consumption. As global fisheries trade has outpaced human population growth to attend demands, resource scarcity, potential higher profits, and weak legislation have encouraged mislabelling. Food fish products represent more than 9% of agricultural exports, so it is important to monitor mislabelling and analyze associated export data. Using DNA barcodes, we carried out molecular identification of products sold as cação (shark), merluza (hake) and linguado (flatfish) in southeastern Brazil. We aimed to determine (*i*) whether umbrella names mask mislabelling and (*ii*) if cheaper species (shark) are less prone to substitution when compared to more expensive or palatable-sounding products such as flatfish and hake. Brazil's export data were obtained for those products from the AliceWeb system to verify how generic names are used for export data, and to check the distribution paths, price, and volume of these products. **Results:** We sequenced 56 shark samples, 52 flatfish samples, and 32 hake samples for the COI-5P barcode sequence. All 140 samples were identified to species level. Six flatfish samples showed mislabelling, and multiple species were included under all three umbrella names (shark, flatfish, and hake). Shark products are exported in large volumes to Japan, China, and Hong Kong, while very few flatfish products were exported to North Korea, and a moderate volume of hake to China. **Significance:** The low mislabelling rates identified may be related to the recent increase of inspection in southeastern Brazilian markets. The use of generic names such as shark also prevents analysis of traded species, making it easy to insert endangered species. A lack of legislation requiring species-specific labelling in both internal markets and trade is a major obstacle to improve consumer-driven sustainable fisheries management.

Ribulose-bisphosphate carboxylase gene (rbcL) sequence-based identification of *Fagonia* spp. of Pakistan

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Background: *Fagonia* is a member of Family Zygophyllaceae. It is found wildly growing in semi-arid and arid areas from the Mediterranean coasts of Africa and Europe to the deserts of the Middle East up to the semi-arid regions of India and Pakistan. Various medicinal properties, most prominently anti-cancer activities, are associated with it. *Fagonia cretica* has colloquially been cited as one of the *Fagonia* species found in Pakistan. Assuring the exact identity of a plant by conventional taxonomic methods alone is demanding. In this study, the species identification of *Fagonia* was done by employing the DNA barcoding technique, which entails sequencing a standard section of DNA as a tool. In plants, various plastid genes have been worked out for barcoding studies; however, the most commonly employed are ITS, matK, and rbcL. Based on the well-characterized features of sequence quality, recoverability, and levels of species discrimination, rbcL was used. **Results:** Eleven plant specimens were collected from various geographical areas of Pakistan. Results for five specimens were only able to be obtained (with 45% success rate). The identification of the sequences by means of the BOLD identification engine indicated that the sequences illustrated maximum similarity (up to 99%) with *F. indica*, with an approximately 96%–99% match in four samples. In one case, it was a 96% match with *F. paulayana*. As *F. paulayana* is not reported in this part of the world and that there is only a single morphological difference between *F. paulayana* and

F. indica, it is concluded that this sample is in fact *F. indica*. **Significance:** This work clearly established that the species of *Fagonia* found in Pakistan is predominantly *F. indica* and not *F. cretica*, whereas the later is incorrectly reported. Moreover, we cannot rule out conventional taxonomy at all; molecular biological approaches should be used in conjunction and not solely.

Using invertebrate DNA metabarcoding to track restoration trajectories of arthropods across two mine site chronosequences

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Background: Ecological restoration is a global endeavour implemented to address biodiversity declines and extensive land degradation. Biomonitoring is a crucial component of restoration, facilitating baseline understanding of ecological condition, and determining restoration trajectories. However, most projects are poorly monitored. Traditional approaches to invertebrate monitoring have been used in a restoration context, but they are underutilised due to the levels of expertise, time, and cost required. Invertebrate DNA metabarcoding has been used to characterise arthropod biodiversity but its application in restoration remains untested. We investigated the invertebrate composition from pitfall traps at two mine site restoration chronosequences in southwestern Australia. **Results:** Invertebrates were profiled using two arthropod COI assays and a plant trnL assay to investigate insect biodiversity and the plants they vector. The assemblages of taxa at the age groups were examined to determine both differences between the sites and the various restoration ages from within each site. The important arthropod taxa that characterise restoration age were also identified. The data revealed significant differences between arthropod communities within both chronosequences. Several significant characteristic taxa were identified for each age within the chronosequence, including ants (Family: Formicidae), springtails (Order: Collembola), and millipedes (Order: Julida). A diverse range of plants was identified from their DNA within the insect samples but did not show any clear patterns between sites or age plots. **Significance:** This study represents the first step in development of DNA-based molecular toolkit for monitoring of ecological restoration projects. Our results demonstrate that a DNA metabarcoding approach, even at an early stage of development, can complement current monitoring practices. Collectively, these data suggest a DNA-based approach will become integral to best-practice restoration monitoring.

InBIO Barcoding Initiative: DNA barcoding Portuguese terrestrial invertebrate biodiversity

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Background: DNA barcoding is an essential tool for biodiversity monitoring and conservation studies. However, its applicability is hindered by the lack of comprehensive reference collections, particularly of invertebrates that are underrepresented in reference databases. In the northwest of the Mediterranean Basin biodiversity hotspot, Portugal holds a unique and diverse fauna. Nevertheless, the vast majority of species remains understudied and underrepresented in DNA barcoding databases. InBIO Barcoding Initiative (IBI) aims to fill the gap regarding Portuguese terrestrial invertebrate taxa. **Results:** By combining fieldwork and networking with taxonomists and ecologists, more than 7000 specimens have already been collected and over 5000 sequenced, covering over 150 families of insects from more than 20 orders. During the development of the IBI reference collection of DNA sequences, many relevant findings on the Portuguese fauna were made. For several groups, a reasonable representation has already been achieved. The state of orders Dermaptera, Hemiptera, Lepidoptera, Mantodea, Mecoptera, Phasmatodea, Odonata, Orthoptera, and Rhaphidioptera will be presented. Major challenges are the hyperdiverse orders such as Coleoptera, Diptera, and Hymenoptera, which represent an enormous part of the invertebrate diversity and have remarkable ecological relevance but are poorly studied in Portugal. For these groups, our focus is the establishment and strengthening of collaborations with taxonomists, and the intensification of the use of sampling techniques that target these taxonomic groups. **Significance:** DNA barcodes of Portuguese invertebrates facilitated the correct identification of enigmatic specimens, namely undocumented species in the region (indigenous and exotic), pinpointed the existence of undescribed species, and allowed linking males and females of sexually dimorphic species. Cryptic diversity was found in several insect groups. The use of high-throughput sequencing to produce the DNA barcodes also allowed us to tackle challenges posed by the existence of nuclear copies. Overall, IBI is expected to become a fundamental tool for biodiversity monitoring in Portugal.

Filling the gap: DNA barcode of Portuguese Dipterans

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Background: Diptera (true flies) are among the most diverse lineages of insects, with over 160 000 described species in the world and over 19 000 present in Europe. Dipterans play an important role in ecosystems at various trophic levels, both as consumers and as prey. Their larvae can be herbivores, scavengers, decomposers, predators, and parasites. The consumption of decaying organic matter, one of the most prevalent feeding behaviours, is of fundamental importance for ecosystems functioning, and several species are economically important crop pests. Despite their ubiquity and remarkable importance, fundamental information on Portuguese Diptera is scarce, with dozens of species still being added to the country inventories each year. Not surprisingly, the application of DNA metabarcoding techniques towards a better understanding of food web complexity in Mediterranean ecosystems, and the identification of trophic relationships relevant for pest management, has been limited by the absence of DNA barcodes. In recent studies, not even half of the operational taxonomic units (OTUs) found were assigned to species level. This project focuses on DNA barcoding Diptera from Portugal and aims to address this knowledge gap. It is conducted within the framework of the InBIO Barcoding Initiative (IBI), a barcoding initiative developed in CIBIO/InBIO that aims to barcode all terrestrial invertebrate groups of Portugal. **Results:** We collected and DNA barcoded more than 200 species of 48 families of dipterans. Genomic DNA was extracted,

and the barcoding mitochondrial COI gene fragment (658 bp) was amplified. DNA barcodes were sequenced using high-throughput sequencing (Illumina). Several species new to the Portuguese fauna were found, including new species to science. **Significance:** While DNA barcoding has significant limitations in dipterans due to their high intra- and interspecific divergence, the documentation of their DNA barcodes in public databases remains of great relevance. The barcoding of Portuguese Diptera is directly assisting the application of DNA metabarcoding techniques.

From phylogenetics to public health: implication of DNA barcoding in determining the genetic diversity of *Oncomelania hupensis quadrasi*, snail intermediate host of *Schistosoma japonicum* in the Philippines

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Background: Schistosomiasis remains a significant public health threat in many tropical and subtropical countries caused by *Schistosoma* trematodes. One species, *S. japonicum*, is zoonotic and is found in the Philippines; they utilize *Oncomelania hupensis quadrasi* snails as their intermediate host. There is limited data on the taxonomic designation of *O. h. quadrasi* in the Philippines as sampling has been limited. **Results:** Provinces with *O. h. quadrasi* were surveyed in the Philippines, and their COI gene sequenced. The NJ tree showed a distinct clade for samples from Surigao del Norte, while the rest of the samples showed a common haplotype. Other subspecies of *O. hupensis* were included in the analysis, indicating that *O. h. quadrasi* is monophyletic and diverged from *O. h. hupensis* (China) with high bootstrap support. Other subspecies appeared to be nonmonophyletic, with *O. h. nosophora*, *O. h. chiui*, and *O. h. tangi* interdigitate with each other. Deeper nodes indicated a high level of statistical support for the divergence of *O. h. quadrasi* from *O. h. hupensis*. *Oncomelania h. robertsoni* formed a distinct clade separate from the other Chinese subspecies. These results from the cox1 data suggest that the subspecies designation of *Oncomelania hupensis* based on geographical location is not well supported. Aside from cox1, other loci, such as the mitochondrial 16S, 12S, and the nuclear ITS, are currently being amplified from the same samples to reveal underlying phylogenetic signal. **Significance:** Proper identification of *O. h. quadrasi* is vital, and elucidation of possible strains is needed for customized snail control efforts imposed in various endemic provinces in the Philippines.

Molecular detection and identification of the invasive Chinese pond mussel *Sinanodonta woodiana* (Bivalvia: Unionidae) from Lake Danao, Leyte and Bato Creek, Oriental Mindoro Philippines using DNA barcoding

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The Chinese pond mussel *Sinanodonta woodiana* (Lea 1834) is a large freshwater dioecious bivalve species of family Unionidae, which is natively distributed in the basins of East Asia. However, due to the introduction of fish stocks infested with the bivalve's parasitic glochidium, numerous reports regarding its presence in other territories have been published. Moreover, its abilities to quickly dominate and invade freshwater habitats pose negative effects on native benthic organisms. Proper identification and detection for the control of the species, however, has been raised due to the resemblance of its shell to some endangered native species. Therefore, to address the limitation of using morphological traits in the identification of *S. woodiana*, we tested the utility of using the cytochrome oxidase I (COI) gene in identifying the species. COI barcodes were acquired from samples of *S. woodiana* collected from Lake Danao, Leyte (n = 13) and Bato Creek, and Oriental Mindoro (n = 5) in the Philippines. These barcodes were subjected to BLAST to confirm the identity of each sample. The gene sequences were then aligned with COI sequences of *S. woodiana* and representatives from family Unionidae acquired from GenBank. In addition, genetic distances between and within species were determined to see if there were overlaps in the within- and between-species distances. **Results:** BLAST searches showed that the sequenced COI gene matched with the gene sequences of *S. woodia*na (>94%) found in GenBank. Phylogenetic analysis also showed that *S. woodiana* from Lake Danao and Bato Creek clustered with other *S. woodiana* isolates from Southeast Asia, forming a tropical invasive lineage. Lastly, pairwise comparisons of all sequences showed no observed overlap in the between- (>0.38%) and within-species genetic distances (0.00%–0.38%). **Significance:** COI barcodes could be crucial for species identification, especially in the case of *S. woodiana*, wherein delineation between native and introduced species using morphological traits may be limited.

River benthos — testing water samples, alcohol filtrates, and insect soups in relation to primer generality and sequence depth

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Background: Environmental DNA (eDNA) and DNA metabarcoding show great promise as tools for monitoring freshwater invertebrate diversity. However, a wide selection of different methodological approaches combined with different genetic markers and bioinformatic analyses requires rigorous testing of how the choice of methods affects estimates of biodiversity in the end. Here we compare DNA metabarcoding of water samples, alcohol filtrate, and homogenization of bulk samples from kick-net sampling in relation to conventional taxonomic analyses of invertebrates in Norwegian rivers. **Results:** Marker choice was highly dependent on sample material. High primer generality worked well on alcohol filtrate and bulk samples. However, for eDNA isolated from water samples, an invertebrate-specific primer was preferable. All sample types produced comparable estimates of biodiversity in relation to conventional taxonomic analyses. **Significance:** Our results suggest that the optimal combination of sample type and marker generality is context-dependent and must be considered in relation to the taxonomic group of interest.

Hidden treasures in your front yard: crustose lichens and lichenicolous fungi in the Norwegian boreo-nemoral and boreal rain forests

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Background: Diverse, highly oceanic forest communities occur in favorable sites along the Norwegian west coast. These boreo-nemoral and boreal rainforests sustain specialized forest epiphytes, including lichens and the lichenicolous fungi inhabiting them. While generally well-studied, particular groups of crustose lichens and the lichenicolous fungi are insufficiently known. In a three-year project funded by the Norwegian Biodiversity Information Centre and in collaboration with NorBOL, extensive mapping and DNA barcoding of these species is currently under way in 32 selected localities stretching from Vest-Agder to Troms. **Results:** The ongoing examination and barcoding of ~6500 observations and 4000 collected specimens so far resulted in approximately 350 identified species including 23, which are

either new to science or new to Norway. Cryptic genetic diversity has been observed in several taxa that is currently under investigation for selected groups in collaboration with qualified taxonomic researchers. As expected, most new taxa have been discovered in the lichenicolous fungi, which are amongst the least-known organisms in Norway. We will present major results, including new species, challenges encountered, and relevant examples of ongoing taxonomic research related to the project. One focus will be on the fungal class Arthoniomycetes, including lichenised, nonlichenised, and lichenicolous species. **Significance:** Norway harbours the only boreonemoral and boreal rainforests in Europe, and the northernmost highly oceanic forest communities in the world. The protection of these endangered habitats has high scientific and societal significance. Profound knowledge on the specialised biological diversity is fundamental and prerequisite for effective conservation efforts. The intensified mapping and DNA barcoding facilitates the discovery of previously unrecognised and cryptic biological diversity. The data collected in this project support future taxonomic studies in insufficiently known organismal groups, and they contribute significantly to existing knowledge on the distribution and the conservation status of species.

Phylogenomic analysis of plastid and mitochondrial genomes improves the resolution of phylogeny of Ericales

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Background: Ericales is a species-rich and highly morphologically heterogeneous order in basal position of the largest Asterids clade of angiosperms. It comprises 22 families, including a holo-parasitic family Mitrastemonaceae. The inter- and intrafamiliar phylogenetic relationships within Ericales remain incompletely resolved, especially for the deep relationships. **Results:** To explore the phylogeny of Ericales, a total of 225 plastid genomes covering all families and most of the subfamilies within Ericales were obtained. A total of 87 mitochondrial sequences covering all the major clades of Ericales were also extracted from genome skimming data. Based on phylogenomic analysis, we firstly placed the Mitrastemonaceae as sister to Primulaceae with high support values. Our results reveal eight major clades of Ericales including (1) balsamiods clade: Marcgraviaceae–(Tetrameristaceae–Balsaminaceae); (2) polemoniods clade: Polemoniaceae–Fouquieriaceae; (3) primuloids clade: ((Primulaceae–Mitrastemonaceae)–Ebenaceae)–Sapotaceae; (4) pentaphylacoids clade: Pentaphylacaceae–Sladeniaceae; (5) ericoids–actinidioids clade: ((Actinidiaceae–Roridulaceae)–Sarraceniaceae)–((Ericaceae–Cyrillaceae)–Clethraceae); (6) styracoids clades: (Styracaceae–Diapensiaceae)–Symplocaceae; (7) Lecythidaceae; and (8) Theaceae. In addition, the balsaminoids clade was supported as the most basal lineage of Ericales. Theaceae was suggested as sister to the styracoids clade with moderate support, and then they are sister to the ericoids-actinidioids clade with high support. Lecythidaceae was suggested as sister to the pentaphylacoids clade with a low support value. Furthermore, the relationships among most of the sampled species were well resolved, with high support values based on the plastid genome. **Significance:** Divergence time estimation demonstrates a mid-Cretaceous origin and rapid radiation after the origination of Ericales, which poses a particularly difficult challenge for phylogenetic inference of the order.

A comparison of COI barcodes, whole mitochondrial genomes and nuclear genomes in detecting post-glacial radiation of a midge

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Background: After the last ice age, marine midges of the genus *Clunio* (Diptera: Chironomidae) have evolved three major ecotypes in northern Europe. *C. marinus* combines circalunar (29.8 days) and circadian rhythms (24 h) to deposit egg clutches on exposed algae during the lowest low tides. The arctic *C. marinus* "Tromsø-type" achieves the same through a circatidal rhythm (12.4 h). Facing the lack of tides in the Baltic Sea, the closely related *C. balticus* deposits egg clutches on the water surface. In order to investigate the evolutionary history of these ecotypes, we sequenced full genomes of 178 male imagoes from eight different *Clunio* populations, covering all three ecotypes. **Results:** From our data we extracted COI barcodes (658 bp) as well as full mitochondrial genomes (15.7 kb) and calculated haplotype networks. COI barcodes have comparatively few polymorphisms, rendering them a good marker for species identification. However, the few polymorphisms that are present in the COI barcodes suggest biogeographic relationships that are contradicting those inferred from the much better-resolved full mitochondrial genomes. Neither COI barcodes nor full mitochondrial genomes cluster according to ecotypes. We currently investigate the differentiation of ecotypes based on >1 million single nucleotide polymorphisms in the nuclear genomes. Comparing nuclear and mitochondrial genomes, we will also test if the discordant evolutionary signals in mitochondrial data could be due to the genus's strong sexual dimorphism, which dramatically limits the dispersal abilities of females. **Significance:** Our results suggest that the use of COI barcodes in biogeographic studies must be carefully evaluated in the light of the biology and ecology of the species under examination.

Integrative taxonomy of nonbiting midges (Diptera: Chironomidae): Skadar Lake versus European barcode library

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Background: We use nonbiting midges (Diptera: Chironomidae) as flagship taxa of freshwater ecology to investigate the interesting but poorly investigated area of the Skadar Lake system (Montenegro and Albania). The area composed of the young Lake Skadar, which originated 1200 a BP, and its old system of springs which originated during Pliocene, is a well-known hotspot of freshwater biodiversity. **Results:** We present the first results achieved during a three-year study aiming to investigate the diversity and origin of the Chironomidae fauna of the Skadar Lake basin. During field campaigns, more than 7300 individuals were collected from different environments (i.e., lake, springs, sublacustrine springs, rivers inflow), and were morphologically identified to 83–86 species; revealing the presence of 56–59 species new to the area, and 6 putative species new to science. For a subset of the collected individuals, a COI barcode library was developed and efficiency of species identification tested using the spider R library. In addition, we tested the efficiency of DNA barcoding for European chironomids, merging the Skadar Lake COI dataset with 9781 publicly available barcode sequences of nonbiting midges, filtered to retain only those identified to species level. The estimated barcoding efficiency confirmed the usefulness of this tool for identification of nonbiting midges. **Significance:** Here, we highlight the importance of linking traditional morphology-based taxonomy with molecular data to improve the knowledge of the fauna of a specific area, and the alpha taxonomy of nonbiting midges. Our results also confirm the usefulness and need for molecular taxonomy in biomonitoring studies of freshwater macroinvertebrates.

The first effort to establish DNA barcode reference library of Gobiidae species for Belarus and Ukraine using DNA barcoding

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Background: At present, the subject of alien species (their impact on the community of native species and ecosystems, risk assessment, and management plans) has a leading position in the world of science, and in the light of global climate change, the number of studies on alien species will increase. Today, in the fish fauna of Belarus there are five Ponto-Caspian Gobiidae migrants: racer-goby, round-goby, tube-goby, stellate tadpole-goby, and monkey-goby. Some aquatic species registered in Belarus are native to Ukraine, and their spread became possible due to transformation of the Dnieper River into a cascade of reservoirs. The aim of the study is to establish a DNA barcode reference library of goby species. **Results:** We obtained DNA barcodes from 30 individuals, which belong to two types of Gobiidae: monkey-goby and tube-goby. Individuals were captured in the territory of Ukraine and Belarus in the waters of the Central Invasion Corridor – Black Sea, a cascade of reservoirs, and the Dnieper and Pripyat Rivers. The received data is uploaded to BOLD (Project Code: GOBYX). **Significance:** This is the first effort to establish a DNA barcode reference library of Gobiidae species for Belarus and Ukraine using DNA barcoding. It will be used as a basis for invasive alien species identification by DNA barcoding, especially of goby specimens in their early developmental stages as well as for monitoring using environmental DNA and metabarcoding. We are working on the DNA barcoding of other goby species of Ukraine and Belarus to complete the library.

A national system for tracing escaped farmed salmon by their DNA and rare mineral deposits

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The number of escaped salmon from Norwegian fish farms has decreased significantly since 2006 as a result of preventive work against escapes; the NYTEK regulation (2011) has improved the technical standard of aquaculture structures, and the organisation OURO (2015) coordinates removal of escaped salmon from rivers. OURO is funded by an annual fee per licence for fish farmers without traceable salmon. Only roughly 10% of all Norwegian farmed salmon can currently be traced back to the owner. In 2014, AquaGen launched the TRACK product, using the salmon's own DNA to trace it back to its owner. A unique combination of parent fish is used to make an egg delivery, which can only be sold to one hatchery. Fifty thousand SNPs are analyzed per parent fish, and the results are stored in a database together with the delivery number. Samples from suspected escapees are analyzed for the same SNPs, the results are checked against the database, and potential escapees can be traced back to their parents and the delivery. Logistic systems are used to narrow down the number of fish farms escapees may come from. As a validation of the TRACK concept, samples from 407 salmon of unknown origin were genotyped. All 220 TRACK fish were correctly assigned to their parents, and none of the 187 nonTRACK fish were falsely assigned. Results from several validation tests show that >99.9% of potential salmon escapees are traced with TRACK. An evaluation of all methods used for tagging and tracing salmon (Tevasvold et al. 2017) recommended a national tracing system based on combining DNA analyses (high density SNP-chip) and rare element analyses of salmon scales. The concept is currently being developed as a national tracing system in Norway. The system can also be used to quantify genetic interactions between farmed and wild salmon.

Long-term plant species turnover in the Alps, inferred from sedaDNA

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Background: Uncertainties in predicted climate change scenarios and the short length of instrumental records provide unclear information about how ecosystems will respond to the effects of climate changes and human pressure. Palaeoecology can be used to better understand the dynamics of past ecosystem changes and composition of plant communities, allowing researchers to improve models of future climate impact on vegetation. High mountain ranges such as the Alps with low human impact are suitable ecosystems to study the local long-term species turnover driven by past climate changes. Previous studies used pollen and macrofossils to infer past vegetation dynamics. However, some inferences were limited by low taxonomic resolution and the preservation of identifiable remains. Conversely, ancient DNA from organisms is also often preserved in the sediment (sedaDNA), which can be detected and analysed with metabarcoding approaches. To investigate the origin and impact of past environmental changes in alpine ecosystems through the Holocene, we performed a multi-proxy reconstruction using sediment cores from lakes of the Western Alps with different human pressures. **Results:** Using sedaDNA, elemental analysis, magnetic susceptibility, and loss-on-ignition data, we reconstructed the long-term vegetation species turnover during the Holocene. We will present the plant community compositions from these analysed records and the general ecosystem shifts inferred from them. We will also pay special attention to the perturbations through human pressure in lakes located close to archaeological settlements. By using the modern DNA sequence database PhyloAlps for the alpine flora of the Alps (4500 species and sub-species), we can bypass the morphological limitations of former palaeobotanical studies and refine taxonomic resolution, often to the species level. **Significance:** The sedaDNA data will allow the inference of more complete species occurrences surrounding the lake catchment, and thus, better assessments of vegetation shifts in past ecosystems, which can be used to better inform future predictions.

Target capture sequence analysis using Angiosperm353 nuclear gene bait set on hemi-parasitic *Euphrasia*, to unravel taxonomic complexities resulting from hybridisation, varying ploidy levels, and repeated evolution of autogamous lineages

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Euphrasia represents a taxonomically complex genus with a bipolar, global distribution, recognised postglacial divergence, and estimates of more than 350 species. Specialisation through hemi-parasitism, varying ploidy levels, extensive hybridisation between species, and repeated evolution of autogamous lineages account for such high diversity. The latest phylogeny (Gusarova et al. 2008) confirms monophyly of the genus; however, it reveals poor resolution and patterns of incongruence between nuclear and chloroplast markers. Suggested solutions to unraveling the genus complexities include thorough next-generation-sequencing (NGS) of nuclear genes, as well as the inclusion of a wider representation of taxa. *Euphrasia* is an exciting group with which to test new NGS techniques and explore the processes of evolutionary diversification because of its unresolved phylogeny, complex DNA patterning, having species of conservation concern, and the disjunct biogeographical patterns and distinct morphological differences between the taxa of the southern and northern

hemispheres. The aim of this study is to test the conserved nuclear loci in the Angiosperm353 gene bait set and its overall suitability for this taxonomically complex group. Ten *Euphrasia* species are used to test the probes, representative of different scales of evolutionary relatedness, covering closely related, known hybrid, and phylogenetically distant taxa from across the three divergent subsects of the genus. Preliminary insights into the level of sequence variation in *Euphrasia* are explored by BLAST analysis of the Angiosperm353 loci against assembled *Euphrasia* genomes. Early analysis results of target capture sequencing using the Angiosperm353 bait set will be presented.

Effect of organic matter sediment content on gut microbiome of gravid *Monoporeia affinis*: an experimental approach

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Background: *Monoporeia affinis* is one of the most abundant macrofaunal species in soft sediments of the northern Baltic Proper and an important bioindicator of environmental stress. *Monoporeia affinis* has a two-year life cycle with its offspring released in February, which is coupled with adults experiencing mass mortality. *Monoporeia affinis* feed mostly on recently deposited organic matter in the spring, and appear to be more opportunistic feeders in the rest of the year. Bacteria present in the digestive tract have been shown to be important in digestion and organism health in many animals. Gut microbes are affected by an organism's environment, influence development, and can indicate stress. A more diverse gut microbiome is proposed to be indicative of better digestive function, enhancing growth, with important consequences at the population level. Can we detect differences in gut microbiomes from anthropogenically stressed populations and how does this affect fecundity and offspring growth and development? In this experiment, we investigated the role of sediment organic matter content on gut microbiomes of gravid *M. affinis* and the effect this has on offspring development and microbiomes. This is the first study of *M. affinis* gut microbiomes related to reproduction of which we are aware. **Results:** While analysis is currently on going, novel data will include gut microbiomes for *M. affinis* individuals and offspring embryo viability and development, and the timing of the offspring release and juvenile growth for two locations in the Baltic Sea. **Significance:** Eutrophication is one of the most pressing problems affecting the Baltic Sea, and the decline of *M. affinis* in the 1990s is attributed to increased eutrophication. Increased nutrients and primary production leads to higher sediment organic matter content. Our results will provide new insights on how the microbiome is related to population dynamics of *M. affinis* and eutrophication influences microbiomes.

DNA barcoding "failure" uncovers two consecutive *Wolbachia*-mediated mitochondrial introgressions in Palearctic swallowtail butterflies

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Background: DNA barcoding performance as a tool for species identification has already been demonstrated beyond doubt for many taxa. Now it is time to change our view and celebrate failures! Once technical problems have been discarded, taxa traditionally considered DNA barcoding failures almost invariably point to instances where taxonomy should be revised and(or) to biological processes worth studying. The European Genetic Map for European Butterflies initiative has provided a sequence library of unprecedented scope and resolution for about 460 species, most of which can be reliably identified by their DNA barcodes. A notable exception is that of the scarce swallowtails (genus *Iphiclides*), which share barcodes despite morphological differences. As a consequence, after more than 180 years since description, *I. feisthamelii* still has a debated status, being often considered a subspecies of *I. podalirius*. Here we aim to elucidate the relationship between the two taxa and the evolutionary processes that led to their separation. **Results:** Our results show that the two taxa clearly differ in male and female genital morphology, male wing UV reflectance, and nDNA, and should be considered as separate species. Two *Wolbachia* strains were found to widely infect the studied samples, apparently explaining the phylogeographic pattern displayed by mtDNA. The available data point towards a *Wolbachia* infection that spread from *I. podalirius* to *I. feisthamelii* and produced a mitochondrial introgression. Currently, a new *Wolbachia* strain is spreading across mainland populations of *I. podalirius*, again mediating a mitochondrial genetic sweep, which has already infected and introgressed *I. feisthamelii* populations in southeastern France. **Significance:** The case of, presumably, two consecutive *Wolbachia*-mediated mitochondrial introgression events further supports the view that infection by this endosymbiont may be frequently related to mito-nuclear discordance in insects. We thus illustrate how DNA barcoding failures shed light on yet poorly documented evolutionary processes.

Macromycetes and plants from the areas contaminated by Sb mining in Slovakia — diversity, toxicity, and remediation potential

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Arsenic and antimony are both widely recognized human carcinogens and declared as toxic pollutants worldwide. Bioremediation using bacteria, fungi, and plants offers promising technological avenues that are highly efficient, environmentally friendly, and cost-effective. In our study, we have focused on arsenic and antimony content in selected species from fungal and plant diversity of the contaminated area. Fungi are the dominating living biomass of soil, but macromycetes have not been widely exploited for the bioremediation of contamination in such environments. Also, they have an advantage over bacteria for the bioremediation of polluted soils owing to their biomass, hyphal networks, and longer life cycles. The advantage of some plant species is their adaptation to contamination by specific biochemical reactions and physiological processes. The absorbed substances are either deposited in the plant tissues and organs or are metabolized to various nontoxic substances (phytotransformation). In some cases, this ability of plants can be used for the purposes of soil remediation. For evaluation of possible utilization of certain species, arsenic and antimony concentrations were measured in selected samples of mycorrhizal (e.g., *Boletus edulis*, *Cantharellus cibarius*, *Russula* spp., *Lactarius* spp., *Cortinarius* spp.), saprotrophic (e.g., *Coprinus* spp.), and parasitic (e.g., *Schyzophyllum commune*, *Armillaria ostoyae*) species of macromycetes, as well as in the reference soil samples and from three selected common plant species: *Cardamine amara*, *Oxalis acetosella*, and *Soldanella marmarossiensis*. In addition, fungal diversity at selected sites near abandoned mines has been evaluated, and more than 130 species were identified by DNA barcoding.

Molecular authentication of Radix Behen Albi (Bahman Sefid) commercial products reveals widespread adulteration

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Background: The Radix Behen Albi, or the roots of *Centaurea behen* L., is used in traditional medicine of southwestern Asian countries as an aphrodisiac, anti-lithiasis, and general tonic. It is available as dried or powdered roots in the local herbal markets. Confirming identity of this

medicinal root using conventional methods is challenging because of lack of diagnostic characters, and market samples are easy to misidentify or adulterate. This study aims to authenticate 13 Radix Behen Albi samples collected from different herbal markets in Iran and to identify its potential adulterants through DNA barcoding. Four DNA regions were used as barcoding markers, nrITS, trnL–trnF spacer, matK, and rbcL. A reference database was compiled using sequences from herbarium voucher specimens and publicly available sequences. **Results:** Among the barcode regions used here, nrITS was the best marker for species identification followed by the trnL–trnF spacer. MatK and rbcL were able to identify samples to family level. This study showed that none of the market samples belonged to the authentic *Centaurea behen* L. Sixty-nine percent of samples was *Cousinia* spp. (Asteraceae), 23% *Korshinskya* spp. (Apiaceae), and 8% *Crambe* spp. (Brassicaceae). This substitution does not only hinder consumers from obtaining the desired medicinal effects of Radix Behen Albi but also raises concerns about the pharmacovigilance of this medicinal root sold in the markets. **Significance:** This study shows the need for monitoring and authentication of crude herbal drugs in the markets of Iran, and that DNA barcoding is a suitable tool for this purpose.

Does eDNA metabarcoding reflect community composition of stream macroinvertebrates at small spatial scales?

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Background: Incorporating environmental DNA (eDNA) metabarcoding into both aquatic community ecology and biological monitoring programs can be advantageous, especially for aquatic insect communities. In addition to overcoming morphological identification constraints (e.g., juvenile or damaged specimens, coarse-level identifications), previous research has suggested that eDNA has the potential to be more sensitive to rare species than traditional kick-net sampling for stream macroinvertebrate communities. Due to the transport of genetic material downstream, it is currently unclear how closely eDNA samples will reflect the diversity of a physical sample at small spatial scales in streams. To assess the congruence of community composition between metabarcoding bulk macroinvertebrates samples and metabarcoding eDNA samples, we collected paired benthic macroinvertebrates via kick-nets and eDNA samples by filtering water on site. Each paired sample was collected from the same location from streams in southern Ontario, Canada. The study sites varied in the intensity of surrounding agricultural land use, allowing us to observe whether the macroinvertebrate community datasets generated by each method respond to the same local and landscape-scale environmental variables (e.g., water chemistry, land use). **Results:** We have observed discrepancies in the community datasets generated by (*i*) coarse-level morphological identification, (*ii*) metabarcoding bulk insect samples, and (*iii*) metabarcoding eDNA samples. Each method yields unique variation in community composition and overall diversity, even though each paired sample was collected at the same location. **Significance:** This is the first study to make direct comparisons of community congruence at the site level between metabarcoding aquatic insect and eDNA samples. Our results also have implications in how these collection methods can best be incorporated into ecological analyses and biomonitoring programs.

African Bushmeat in Brussels: high prices and high levels of misidentification

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Background: Notwithstanding the prohibition at national and European Union levels to import individual consignments of meat (products), various publications report that large quantities of meat, including bushmeat, are entering Europe via its international airports, without authorization or certification. These studies suggest an organized market for African bushmeat in Europe. In order to get a first idea of the nature, extent, and value of African bushmeat trade in Brussels, 16 travellers from Central African countries living in Brussels were interviewed. In addition, 15 pieces of bushmeat were bought in African grocery shops to explore (*i*) which species are being sold, (*ii*) if these are endangered or legally protected (e.g., CITES), and (*iii*) the price of bushmeat in Brussels. **Results:** The interviews revealed that "food from home" is considered superior and that, notwithstanding the awareness of the rules and policies, African food products are often imported. The interviews also showed that apart from the authenticity and flavour arguments, bushmeat trafficking is seen as a way to make money or pay the travel expenses, while customs control is perceived as a lottery without much risk. The bushmeat pieces were identified using DNA barcoding. They involved nine mammal species, and seven of the 15 pieces were sold under a wrong species name. None of the nine species were endangered (IUCN Red List), but four species were CITES-listed. The maximum price of bushmeat in Brussels is twice the Belgian market price of a piece of premium livestock, but does not seem to be related to the species being sold. **Significance:** This study confirms that various types of bushmeat, including CITES-listed species, are available for sale in Brussels. The high prices confirm the luxury status of the product, while DNA-based identifications demonstrate that bushmeat 7is often mislabelled.

Seafood on the Belgian market: do you get what you are paying for?

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Background: Compared to other European countries, Belgian customers are moderate consumers of fish and seafood products. The Belgian fishery and aquaculture sectors, however, are relatively small, and a large part (81% in 2016) of the fish and seafood consumed in Belgium is imported, half of which originates from outside Europe. Since the scale of the international trade increases the potential for (un)intentional misidentification and(or) deliberate fraud through species substitution, we examine the correctness of the labelling of a number of seafood products on the Belgian market. The study focusses on shellfish, cephalopods, and crayfish. **Results:** Samples were identified by DNA barcoding, and the resulting DNA-based identifications were compared with the information on the commercial labels. Preliminary results indicate that there are large differences in the frequency of mislabelling between the different taxa investigated, as well as when considering packaging (sold individually or as part of a seafood cocktail) or treatment (e.g., fresh, frozen, cooked). **Significance:** More stringent regulation on foodstuff labelling is supposed to protect consumers and the seafood industry from (un)intentional mislabelling. However, several studies worldwide indicate that (un)intentional misidentification and(or) deliberate fraud through species substitution is common practice, especially for processed products that lack characterizing morphological features. It also seems that the scale as well as the product most prone to mislabelling differs by country. The present survey, therefore, aims to identify the level of mislabelling for seafood products sold in Belgium.

Barcoding organisms and tissues of policy concern: experiences from three years of BopCo

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The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo) is jointly run by the Royal Belgian Institute of Natural Sciences (RBINS) and the Royal Museum for Central Africa (RMCA), and is part of the Belgian federal contribution to the European Research Infrastructure Consortium LifeWatch. As such, BopCo is financed by the Belgian Science Policy Office. BopCo aims to act as a focal point for the identification of biological materials of policy concern for all stakeholders who deal with such samples and who are in need of an accurate identification. Therefore, BopCo provides identifications upon request or connects individuals with taxonomic experts who possess the required know-how and means to identify the sample(s) and who can provide background information on the concerned organisms. Morphological species traits, DNA barcoding and, if needed or relevant, other DNA-based technologies are being used, alone or in combination, to provide reliable taxonomic identifications. A prerequisite to use DNA barcoding is the availability of comprehensive and reliable reference barcode libraries. Therefore, BopCo also contributes to populating DNA barcode databases of taxa of policy concern. To this end, BopCo produces new DNA barcodes, either at its own initiative or in collaboration with research institutes, governmental organisations, universities, and others. The BopCo project was first announced at the 4th iBOL Conference in 2011, but only started running at the end of 2015. Now, a little over three years into the project, we present some of our output and experiences, examples of identification requests, identification projects, and collaborative activities, including cases of several policy concern categories like endangered and protected species, agricultural pest species, human and veterinary disease organisms and their vectors, organisms of the food chain, species of forensic interest, and invasive species.

Expanding the reference library of a Neotropical megadiverse nation

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Background: Bioscanning megadiverse countries is the main challenge to successfully accomplish the planetary biodiversity mission. Huge efforts are being made to fill the gaps of genetic information in Colombia that has been largely underexplored, partly due to internal conflict. From 2016 to 2018 after signing the peace agreement with the FARC guerilla, we conducted five biological expeditions in previously unexplored regions of cloud forest, páramo, and savannah ecosystems with a team of taxonomists and molecular ecologists that collected plants, fungi, vertebrate, and invertebrate specimens. For insects in particular we set nine Malaise traps during a week. **Results:** We generated 3802 DNA barcodes from 3234 specimens evenly distributed between Arthropoda, Chordata, and Magnoliophyta, doubling the public amount of barcodes from Colombia. We barcoded 21 new species of plants, fungi, amphibians, fish, and insects and generated the first genetic record for 45 species of birds, fish, amphibians, and mammals. For Malaise traps, we Sanger sequenced 686 specimens that correspond to 354 unique BINS. In parallel, we seek to recover a barcoding-compatible COI fragment from 2223 Malaise traps samples using Illumina MiSeq sequencing technology. So far, we have recovered 65% samples with a trustable COI sequence from MiSeq, but further bioinformatics efforts are ongoing to raise this statistic. **Significance:** At least 30% of the generated sequences correspond to the first genetic data for the species in Colombia, filling a major geographic gap in Neotropical research. This project showcases the benefits of the post-conflict era and paves the road for future expeditions that link taxonomic expertise, genetic pipelines, and community's participation, resulting in an increase of biodiversity knowledge and the enrichment of reference libraries.

Assessment and status of southern African marine mammal collections

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Museum collections are an invaluable data source, if properly accessible and available to the local and global research community. The Natural Science Collections Facility (NSCF) is a recently formed distributed network of South African Institutions all working towards securing and making collections accessible that are curated in line with global best practices and standards. The local cetacean collection comprises of those housed at Iziko South African Museums, Bayworld Port Elizabeth Museum (PEM), and other miscellaneous collections. Data holdings include University of Pretoria's Mammal Research Institute, Iziko, and PEM SPECIFY databases, Department of Environmental Affairs, Sea Search Research and Conservation, and the Namibian Dolphin Project databases; all based on different standards. The viability of using Iziko holdings to conduct genetic analyses is currently unknown. Moreover, a comprehensive assessment, including inventory and curatorial status of all cetacean material, has not been performed, and there is significant duplication between the databases. As a consequence of working in silos, the associated databases do not meet international standards (Darwin Core) and are not interoperable with each other. This NSCF project is working towards inventorying all cetacean collections housed at South African Institutions, collating, and standardizing the diverse databases with the primary aim of assessing and making accessible all tissue viable for genetic research (DNA barcoding, phylogenetics, and phylogeographic studies). The inventory will ensure samples are available from a wide variety of locations, an important strategy given that some species can be difficult to sample depending on their distribution or if highly threatened in the wild. Data verification and quality control of the collection will promote research, including the generation of scientific knowledge from a wide range of questions on taxonomy, population genetic structure, phylogeography, and cryptic speciation that will add value to current and future conservation management and development of marine policies in South Africa.

Retracing origins of Pacific leaf-cutter bees (Megachilidae)

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Background: Bees represent primary pollinators in almost all terrestrial ecosystems and contribute a major service to the production of agriculture. But our understanding of even fundamental aspects of their diversity and distribution remains piecemeal. Islands typically harbour reduced species richness that may enable broader host ranges than continental systems, meaning few pollinators can impact many plants. Records of bees across the Pacific indicate very low species diversity, despite otherwise rich biota. Megachilid bees comprise a large proportion of the bee fauna in most Pacific archipelagos, but, due to a preference for nesting within wood, many species likely span multiple island groups. **Results:** We sequenced mitochondrial DNA (COI) representatives from Australia, PNG, Solomon Islands, Vanuatu, Fiji, Samoa, and Micronesia to indicate multiple recent introductions into the Pacific, likely from Southeast Asia and Australia. These results indicate that much of the Pacific bee

fauna arrived only very recently, meaning their role in the evolution of biodiverse island floras was likely limited. **Significance:** This has very wide implications for understanding Pacific plant–pollinator relationships, where ancient pollination mutualisms may be at threat from recent bee introductions. However, if recently arrived species are found to be effective crop pollinators, their distribution across multiple archipelagos may enable management strategies to be applied regionally.

Molecular study of *Chaetozone* Malmgren (Annelida, Polychaeta) reveals hidden diversity of a common benthic polychaete

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Background: The polychaetes of the family Cirratulidae (Annelida) are common inhabitants in offshore benthic sediments and considered as an important group in environmental monitoring. Amongst them, the genus *Chaetozone* is the most species-diverse worldwide. Seven species of *Chaetozone* have been recorded in Norway, although these records should be considered cautiously as species delineation is challenging with morphological means. In order to determine the number of species present in Norway and their distribution, over 200 specimens from Norwegian and adjacent waters were DNA sequenced (the universal mitochondrial barcoding region COI, and D1–D2 regions of the nuclear 28S rDNA) and datasets investigated after phylogenetic and species delimitation analyses such as ABGD, mPTP, and GMYC. These molecular analyses were used as a frame to re-examine the morphological diagnostic features of each of the species. **Results:** Over 100 new COI barcodes are obtained, and a total of 12 species are recovered in the analyses. These include sequences from specimens of the type locality of the type species of the genus, *Chaetozone setosa*, and its distribution was confirmed to be limited (in Norway) to Arctic waters. The morphology and nomenclature of all species are discussed. **Significance:** This is a first molecular approach to resolve the species delineation, evolutionary relationships, and geographic structure of members of *Chaetozone*, a genus whose taxonomy has proven to be difficult. It provides a tool for both molecular and morphological identification and demonstrates the considerable underestimation of the diversity of *Chaetozone*, in the North East Atlantic. This also gives a taste of what is to be expected for the rest of the elusive systematics in the family Cirratulidae.

A study on the identification of *Epimedium* species

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Background: Epimedii Wushanensis Folium, derived from the dried leaves of *Epimedium wushanense*, has been widely used in traditional Chinese medicine (TCM) for a long time to nourish the kidneys and reinforce the Yang. However, *E. wushanense* is easily confused with its closely related species, namely *E. pseudowushanense*, *E. chlorandrum*, *E. mikinorii*, and *E. borealiguizhouense*, due to their highly similar morphological characteristics, thus leading to potential safety risks. **Results:** In this study, the chloroplast (cp) genomes of the five *Epimedium* species were sequenced. The cp genomes of *E. wushanense* and its relative species are a typical circular tetramerous structure. A total of 112 genes were identified from the five cp genomes, including 78 protein-coding, 30 tRNA, and 4 rRNA genes. The maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees constructed based on the complete cp genomes demonstrated that *E. wushanense* can be separated from its closely related species. **Significance:** The cp genome data contribute valuable information for identifying *E. wushanense* and its relative species, which also provide new evidence for classifying *Epimedium* species.

Multi-locus DNA metabarcoding of diet provides new insights into the ecology and trophic interactions of a keystone herbivore species

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Background: Determining trophic interactions is key to understanding ecosystem functioning and provides the baseline knowledge necessary for implementation of ecosystem conservation and management measures. Reindeer is one of the last remnants of the Beringian megafauna, and a keystone species in arctic ecosystems. Being the most abundant large herbivorous species in the Arctic, reindeer provide essential ecosystem services to indigenous peoples and have a complex network of biotic interactions with primary producers (plants and lichens), predators, and humans. To sustain reindeer herding and tundra ecosystems, detailed knowledge about reindeer nutritional base is required, as well as identification of the main drivers and interactions involved in the reindeer-human system. In the ongoing REININ project, we analyzed more than 1000 reindeer faecal samples from Svalbard, northern Norway, and the Yamal Peninsula where different, or no, management regimes are implemented. In all geographic areas, fecal samples were collected from both reindeer and sympatric herbivore species along with vegetation records. **Results:** We metabarcoded faecal samples at multiple loci including the trnL intron for plants and bryophytes, ITS2 for plants and fungi (including lichens), and 18S (to enable screening of eukaryotic taxa). Application of multi-locus DNA-barcode libraries provided a trade-off between broad taxonomic coverage and greater taxonomic resolution. We describe and discuss (*i*) complex diet content consisting of all major plant groups and lichenicolous fungi, (*ii*) seasonal and geographic variation in diet of reindeer, and (*iii*) overlap with other herbivorous species. **Significance:** Previously, reindeer diet composition has often been based on coarse categorizations of forage types or on small samples taken over short time periods. Such data do not allow exhaustive taxonomic characterization of the realized diet, which is important to quantify diet breadth and overlap. Presented results will provide the first comprehensive DNA metabarcoding dataset about herbivore trophic interactions in the Arctic.

Extreme genetic diversity of Collembola among subterranean calcretes in arid Australia

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Background: The island hypothesis for calcretes of the Yilgarn region in western Australia has held true for many stygobitic species that are ostensibly "trapped" evolutionarily within isolated aquifers due to their aquatic life-styles. However, very little is known about the distribution of terrestrial invertebrate species associated with the calcretes. Here, we use troglobitic Collembola from the Yilgarn calcretes to test

the hypothesis that troglobitic species (i.e., those inhabiting the unsaturated zone of calcretes) are restricted in their distribution and represent reciprocally monophyletic and endemic lineages. **Results:** We used the mtDNA cytochrome c oxidase subunit 1 (COI) gene from 183 individuals to reconstruct the phylogenetic history of *Pseudosinella* Schäffer (Collembola, Lepidocyrtidae) from 10 calcretes. These calcretes represent only 5% of the total possible calcretes in the Yilgarn region, yet we show that their diversity for subterranean Collembola comprises up to 29 putative species. Further, at a regional scale, multiple levels of genetic diversity exist in *Pseudosinella*: old divergences between major lineages; some lineages restricted to individual calcretes, while others are unrestricted in their distribution; and, recent mixing of haplotypes between populations within calcretes, even over large distances. **Significance:** The implications of our results suggest we have only just scratched the surface of the diversity of Collembola in the Australian arid zone. The results also indicate that many of the (provisionally) troglobitic Collembola have probably been impacted by climatic oscillations on the surface, facilitating their dispersal across the landscape. These observations are indicative of an evolutionary history for troglobitic Collembola in the Yilgarn that is more complex than previously observed for the aquatic fauna that are protected within the confines of the calcretes.

Limiting PCR biases and limitations due to PCR-based metabarcoding short length fragments: a comparison with two possible alternatives, capture by hybridisation and nanopore sequencing

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Metabarcoding of environmental DNA (eDNA) opened large prospects for the future biomonitoring of marine environments. We developed a molecular pipeline for metabarcoding standardized mitochondrial COI and ribosomal 16S, 18S barcodes, allowing inventory of biodiversity from bacteria to vertebrates. The recently improved bioinformatics pipelines allow more conservative and reliable assessments. Yet, metabarcoding still relies on polymerase chain reaction (PCR) of relatively short fragments, resulting in taxonomic biases for some important lineages, which are still very partly revealed by universal metabarcoding primers. The short barcode lengths also limit the reconstruction of robust phylogenies. Here, we present tests to adapt sequence hybridization capture methods for inventorying biodiversity in deep-sea sediment samples. This PCR-free method was expected to broaden the spectra of lineages captured in inventories and obtain longer DNA fragments, resulting in better phylogenetic reconstruction and enhanced taxonomic resolution, an essential improvement for environments still largely underrepresented in public databases. Results of the first tests on samples from a diversity of ecosystems (Abyssal plain, seamounts, hydrothermal vents, etc.) partly fulfilled those expectations, yet at the price of a complex bioinformatics process of assemblage. We thus also compared our results with the long sequencing technology offered by Oxford Nanopore with the MinION device, which also has the advantage of allowing real-time analysis at sea. We will present a synthesis of the results obtained with the three methods (*i*) metabarcoding, (*ii*) capture by hybridization, and (*iii*) MinION sequencing, and offer a set of criteria to choose the best-adapted method depending on the main goal for biodiversity inventories.

Can environmental DNA metabarcoding stand the test of time?

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Background: DNA metabarcoding relies on next-generation sequencing analysis of marker genes such as the COI DNA barcode to provide biodiversity data directly from environmental samples. Given the need for biodiversity information in various ecological investigations from biomonitoring to detection of rare/endangered species, DNA metabarcoding has gained significant momentum. However, the use of this approach for long-term and large-scale studies of ecosystem dynamics has not been sufficiently demonstrated. **Results:** Using sequence data collected from benthos in various watersheds across Canada, we show the robustness of DNA metabarcoding (both molecular and bioinformatics methods) in providing biodiversity data for spatial ecosystem analysis. We also use data from seven years of sampling to demonstrate the capacity of DNA metabarcoding to tease apart seasonal and annual changes in biodiversity. **Significance:** Given rapid advances in HTS technology and bioinformatics approaches, DNA metabarcoding methodologies require careful optimization and testing to successfully operationalize them for scientific and socio-economic applications. This is especially important for applying DNA metabarcoding in biomonitoring and environmental impact assessment programs where ecosystem states are examined over time and space and in linkage to various anthropogenic activities.

Curating reference libraries for regulatory applications of DNA barcoding

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Background: DNA-based tools for species identification have a variety of socio-economically relevant applications, including endangered wildlife, food and natural health products, regulated plant pests, and animal parasites that are vectors of zoonotic disease. The utility of DNA barcoding has been demonstrated for all of these applications, but regulatory uptake requires standard operating procedures (SOPs) tied to curated reference sequence libraries in order to provide consistent, repeatable results when attempting to identify an unknown sample based on its DNA sequence. Due to their ever-expanding nature, public databases do not consistently meet this need because they may yield different matches to the same query sequence over time. Database annotation and curation protocols are needed. **Results:** Although GenBank and BOLD are commonly used tools for matching an unknown sequence to infer a species-level identification in academic settings, varied regulatory agencies are developing their own curated reference sequence libraries deemed fit-for-purpose in molecular diagnostic applications. Notable examples include the Quarantine Barcode of Life (QBOL) library hosted by the European Plant Protection Organization (EPPO) and the Reference Fish Encyclopedia hosted by the US Food and Drug Organization (FDA). Although useful, these data sets contain relatively limited sample sizes that may exaggerate an apparent barcode gap. Here, we describe a hybrid approach that both designates exemplar sequence(s) linked to voucher specimens and also includes other key metrics such as proportional barcode representation of a taxonomic target group and range of intraspecific variation as estimated from available public data. **Significance:** This curatorial approach represents a conceptual bridge between public databases and regulatory data sets that is designed to enhance both academic and regulatory applications of barcoding.

Barcoding mammal field collections using PacBio

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Background: Cryptic diversity is a theme in tropical mammalogy, leading to difficulty in correctly identifying species in the field. Due to the cost restrictions of sequencing, many researchers will use barcoding to identify one or two unknown specimens and use those as exemplars to identify a much larger series morphologically. **Results:** We

used PacBio sequencing with long barcodes (full length Cytb) to identify all specimens collected on three field collections to Ecuador and Panama. In the process, we identified at least one unknown species. **Significance:** NextGen sequencing approaches provide significant cost reduction at the loss of sequencing shorter fragments. PacBio provides multiplex ability with long read chemistry. We discuss pros and cons to this approach as well as future potential.

Species diversity mapping using metabaroding data

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Background: The importance of arthropod biodiversity to maintain agricultural ecosystem services, functions, and stability is widely accepted. However, understanding farmland biodiversity is hampered by the lack of temporal and spatial distribution information of species richness due to labour-intensive and time-consuming field measurements. A new generation of computationally demanding models could greatly enhance our capacity to monitor and predict ecosystem change at larger scales, but they demand better approaches for gathering and synthesizing biodiversity data. The comprehensive ecosystem data required by these models is currently unavailable because traditional approaches only examine certain biotic compartments and then only at scales of a few metres. **Results:** In summer 2017, we deployed a series of Malaise traps (SLAM type) across various agricultural plots with four crop types (alfalfa, soy, wheat, corn) at University research stations. Each plot hosted five traps at specific locations. Specimens were metabarcoded to provide weekly assessments of community composition. We used the resulting data to develop a species diversity mapping script for R. Multiple operational taxonomic unit (OUT) tables representative of multiple collection events can be run simultaneously by the script. Outputs for each sampling event are used to produce standard shapefiles for use in GIS packages as well as individual map plots that can be used to assemble animated GIFs that could be used to visualize temporal variation. **Significance:** It is rather difficult to identify all the species present in a location let alone to quantify their responses to environmental change. The combination of high-throughput DNA sequencing with the statistical modelling we developed makes it possible to scale up from data-rich but finite sets of point samples to spatially continuous biodiversity maps, which we can use to generate summary statistics to communicate trends to farmers, decision-makers, and the general public.

Investigating host–plant relationships of Peruvian Lepidoptera from fogged caterpillars: DNA barcoding and gut content analysis

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Background: Knowledge on host–plant relationships of tropical Lepidoptera is still scarce despite their importance for agriculture, forestry, ecological research, etc. **Results:** Canopy-fogging samples were taken from 47 trees near the Panguana station in Peruvian western Amazonia. Out of the alcohol-preserved samples we selected 130 Lepidoptera larvae, which were subsequently dried, photographed, and databased. One vertically cut central segment of each larva was submitted to Sanger sequencing of the COI DNA barcode fragment with Lepidoptera standard primers for species identification. All data and photographs are deposited in BOLD. Sequencing of the COI barcode gene was successful for 115 larvae (88%). A BLAST search on BOLD resulted in 56 identifications to species level (49%), 33 to genus level (29%), and 23 to family level (20%). For just three sequences no family match was suggested. Altogether, the 115 sequences belong to 90 different lepidopteran species-clusters (BINs). For 10 larvae from six target trees additional tissue samples of the gut contents were analysed with four plant-specific primers (psbA, rbcLa, matK, ITS2) in a Next-Generation-Sequencing (NGS) approach to discriminate feeding on the fogged and pre-identified target tree from feeding on neighbouring trees or alternative feeding on lianes, epiphytes, algae, mosses, or lichens. Reliable sequences were obtained for just two of the four markers, psbA and rbcLA. **Significance:** The results preliminarily suggest that (*i*) the scientific approach is working and (*ii*) alternative feeding plays an important role in Lepidoptera of the Peruvian Amazon. A subsequent, more comprehensive study has been started to analyse host–plant relationships of fogged Peruvian caterpillars through NGS-based gut content analysis using rbcLa, trnH–trnF, and ITS2.

BIOSCAN — Revealing species diversity, interactions, and dynamics

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DNA sequencers are doubling their output every nine months, reinforcing their ability to support audacious projects. What happens when you employ them to address the key dimensions of biodiversity—the number of species, their interactions, and their distributions? We will gain a species count for the planet. We will learn to read the symbiome, the complex assemblage of parasites, commensals, mutualists, and microbes that interact with every species. We will track shifts in the distribution and abundance of these species on a global scale. Quantifying species diversity, revealing symbiomes, tracking species dynamics; those are the goals of BIOSCAN, a seven-year, $180M project launched by the International Barcode of Life Consortium in June 2019. By deep sequencing 15 million single specimens, a massive number of new species will be registered, and their previously dark interactions will be revealed. By metabarcoding 100 000 bulk collections from 2000 sites spanning half the world's ecoregions, BIOSCAN will lay the foundation for an earth observation system for biodiversity. Although it will not illuminate all species or fully reveal their dynamics and interactions, BIOSCAN is the foundation for a 20-year mission that will achieve these goals.

Extended phylogeny of the lichenized algal genus *Trebouxia* (Trebouxiophyceae, Chlorophyta): insights from chemosystematics and symbiotic pattern of Icelandic cetrarioid lichens

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Background: Lichen symbiont interactions (e.g., myco- and photobionts) play a crucial role in the high environmental tolerance of lichens, in which neither symbiont can hardly survive independently. The characterization of fungal-algal association patterns is essential to understand their interactions and to elucidate the symbiotic mechanisms. Lichens are prolific producers of structurally unique secondary metabolites, which are of ecological and taxonomic importance. **Results:** This study investigated fungal-algal association patterns in Icelandic cetrarioid lichens (168 specimens of 13 cetrarioid taxa) using a multi-locus phylogenetic framework, including fungal nrITS, MCM7, mtSSU, and RPB1 and algal nrITS, nrLSU, rbcL, and mtCOXII data. Lichen metabolite profiles were assessed using ultrahigh-performance liquid chromatography-mass spectrometry. Our chemical results revealed that *Cetrariella delisei* is the only Icelandic cetrarioid lichen producing depsides, but not aliphatic lactones, dibenzofurans, or depsidones. Most Icelandic cetrarioid lichenized fungi were found to be specifically associated to the known *Trebouxia* clade S (*T. simplex*/*suecica* group), while the lichen-forming fungus *C. delisei* forms symbiosis with a previously unrecognized *Trebouxia* lineage here provisionally named as the D clade. This new *Trebouxia* lineage is supported by Maximum Likelihood and Bayesian phylogenetic analyses using all four included algal loci. We suggest a preliminary num-

ber of six operational taxonomic units (OTUs) in this new clade using different species delimitation methods (i.e., ABGD and bPTP). The phylogeny of the lichenized algal genus *Trebouxia* is re-constructed and updated. **Significance:** Our symbiotic association pattern results also demonstrate the importance of algal partners in shaping lichen symbiosis with the example of Icelandic *C. delisei*, which challenges a previous hypothesis suggesting a determining role of fungal partners.

Refining bioinformatic filters for processing metabarcoding data from ultra-low concentration (ancient) environmental DNA

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Background: The ultra-low occurrence of template DNA molecules is a feature of ancient environmental DNA (eDNA) and some eDNA extracts, such as those derived from water samples. PCR-based metabarcoding of such extracts can require 40–50 cycles of PCR to ensure that templates are amplified to the concentrations required for sequencing. These applications are therefore more sensitive to contamination and the impacts of PCR errors and biases, as compared to the metabarcoding of other eDNA templates. This is particularly apparent for studies that target short markers with highly complete regional reference libraries (such as the vascular plant chloroplast p6-loop), as taxonomic assignments may differ based on a single base pair difference. Here we use a combination of synthetic positive controls and a refined bioinformatic pipeline to minimise the impacts of contamination and PCR errors/biases. Such refinements include correcting for index hopping between libraries, the detection of potential homopolymer errors, and correcting for differences in sequencing depth between PCR replicates. **Results:** We show that each of these refinements reduces noise in the data and therefore allows for rare amplicons to be more confidently detected. The identification of amplicons with long homopolymer runs, which are prone to polymerase error, has also allowed for the detection and removal of taxonomic assignments that were unexpected based on ecology and(or) biogeography. **Significance:** The optimisation of bioinformatic analysis for sensitive metabarcoding applications better leverages these data and allows for more robust and in-depth inferences at no additional cost.

Analyses of fish, amphibian, and mussel communities using eDNA metabarcoding versus traditional methods

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Background: Sound environmental management decisions—in accordance with the EU WFD for aquatic ecosystems—mainly depend on reliable species presence and distribution data. Species under water are out of sight and are difficult to monitor and detect using traditional methods. Here, we compare the outcome of historical records of fish, amphibian, and mussel species in 100+ freshwater bodies (lakes, dams, and rivers) with environmental DNA (eDNA) metabarcoding data retrieved once to twice at each site between 2016 and 2019. **Results:** The analysis revealed that eDNA detects between 50% and 100% more species than traditional survey methods. This was especially true for rare, elusive, and newly invasive species. The species presence from eDNA was confirmed by sport-fishing records, fishing, water binocular, snorkelling, and interviews. **Significance:** A sound eDNA pipeline including data collection, extraction, choice of markers, and metabarcoding pipeline underpins the potential for a future paradigm shift for environmental monitoring over large geographic areas.

Using eDNA to monitor reintroduction success and distribution of an indicator fish species in a remediated stream

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Background: Monitoring of specific indicator species in stream ecosystems is essential to track changes in their ecological status. The traditional monitoring approach uses complex and invasive methods such as electrofishing or kick sampling. A more modern and sensitive approach based on environmental DNA (eDNA) and DNA barcoding could serve as a complement. Organismal eDNA is released into the water by faeces, saliva, urine, or epidermal cells and can be extracted from small samples to be analysed using species-specific DNA markers in order to confirm presence and absence of target species. **Results:** We developed an assay for eDNA-based monitoring of the Rhine sculpin (*Cottus rhenanus*), an indicator species used to determine water quality, water body structure, and passability. The assay was applied across high spatial and temporal resolution after reintroduction of the species into a remediated stream to monitor reintroduction success and distribution of the Rhine sculpin as well as remediation success and ecological status of the stream. For comparison, electrofishing was carried out once in addition to eDNA sampling. Establishment and distribution of the species was confirmed by both eDNA and electrofishing, thereby indicating a successful stream remediation and a good ecological status. Moreover, it was shown that the detection via eDNA was more sensitive than traditional electrofishing, as the former confirmed the species' distribution across a putative barrier which was overlooked by the latter. **Significance:** Our results indicate a much higher distribution potential of the Rhine sculpin than previously thought, both with respect to speed and the capability to cross barriers. Moreover, it was shown that complementing traditional methods with eDNA is recommended and that eDNA data are applicable for freshwater monitoring, remediation, and future water management.

An integrated approach to untangling vector, host, and pathogen interactions (xenosurveillance) in mosquitoes (Diptera: Culicidae) from five communities close to Lacandone Jungle, Mexico

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Background: The females of most mosquito species require a bloodmeal for egg maturation, and can transmit pathogens during this process. Therefore, rapid identification and delineation of mosquito species, as well as identification of the species that provided blood and of the pathogens within the bloodmeal, are pivotal to disentangling vector–host–pathogen interactions (xenosurveillance), which can lead to a better understanding of pathogen transmission and, ultimately, control. In this paper, we firstly employed DNA barcoding to identify mosquito species at five sites in Chiapas State, Mexico. In addition, we applied second-generation sequencing (Ion Torrent PGM) to identify the vertebrate species present in their bloodmeal, and used generic qRT PCR for the detection of arboviruses. **Results:** Morphological study revealed 56 mosquito species in 12 genera, including three spe-

cies new to Mexico, and 10 new to Chiapas State. The DNA barcode analysis of 183 specimens representing 34 of these species revealed that most specimens were placed to the species assigned by morphology, although some inconsistencies were found in *Wyeomyia* and *Trichoprospopon*. We could identify the source species for the blood meal in about 40% of the specimens. These results indicated that *Aedes angustivittatus* fed on ducks, while *Psorophora albipes* targeted humans. By comparison, *Culex quinquefasciatus* fed on diverse hosts (chicken, human, turkey, Mexican grackle). No arboviruses were detected in DNA extracts from single blood-fed specimens or from extracts prepared from large numbers of specimens. **Significance:** By generating a barcode library for mosquito species from Lacandone Jungle, Mexico, this study confirms the utility of DNA barcoding in vector surveilliance. In addition, it demonstrates that Ion Torrent technology is a fast and inexpensive option for the identification of host DNA within the bloodmeal.

Building barcode libraries of Norwegian microcrustacea

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Background: Microcrustacean groups are highly diverse, and are important elements in aquatic food webs (pelagic as well as benthic). Here, we summarize progress in building barcode libraries for microcrustaceans (copepods, cladocerans, and ostracods) in freshwater and marine waters of Norway, based on two faunal mapping projects supported by the Norwegian Biodiversity Information Centre and NorBOL. **Results:** The COPCLAD project focused on marine pelagic copepods and cladocerans, collected in fjords as well as open seas. 475 specimens were submitted, resulting in barcodes for 61 nominal species. The BARCRUST project included freshwater cladocerans, copepods, and ostracods, collected all over mainland Norway and some Arctic regions. 475 submitted specimens resulted in barcodes for 142 nominal species. In both projects several cryptic taxa were shown to occur, highlighting species groups in need of taxonomic revision. Examples include the marine pelagic copepod genera *Microsetella* and *Microcalanus*, and the freshwater genera *Eurycercus* (Cladocera) and *Bradleystrandesia* (Ostracoda). **Significance:** Microcrustaceans are important elements in environmental monitoring as well as ecological research in marine as well as fresh waters. Identification by traditional means remains time consuming and costly. Moreover, much of their life-cycle is spent in unidentifiable juvenile instars, which implies that major fractions of aquatic communities remain unknown in most samples. DNA-based methods hold promise to provide faster results at lower costs, and also substantially higher precision in environmental monitoring and biodiversity research, provided that comprehensive libraries are available. Our efforts are intended to contribute to these libraries, to expand our knowledge of species richness and distribution, and to pin-point species complexes that need further resolution.

Community phylogenies of ectomycorrhizal fungi from Guineo-Soudanian ecozone of Benin (West Africa)

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Background: Mycology has experienced a rapid development during the last two decades through the application of molecular techniques and phylogenetics to fungal taxonomy, ecology, and evolution. Many fungal species display a limited number of morphological and anatomical characters, making species demarcation difficult. To get a clear picture of fungal diversity and community phylogenetics in Benin, systematic sampling of fruit bodies of ectomycorrhizal fungi was carried out in "Oueme-superieur" forest reserve. DNA was extracted from representative specimens of each morphological species using either the QuiaGen DNeasy Plant Mini kit or a protocol of cryogenic disruption followed by extraction in CTAB buffer. The internal transcribed spacer (ITS) region of the rDNA, LSU, and RPB2 was amplified by PCR. Phylogenetic tree inference was performed using Maximum Likelihood (ML) and rapid bootstrapping on XSEDE with RAxML. To know whether the species harvested in Benin are closer to the African species of the tropical region or species from other regions, an analysis in R with the Picante package was performed. **Results:** A total of 9 species in Amanitaceae, 3 in Boletaceae, 1 in Cantharellaceae, 1 in *Cortinarius*, 2 in Inocybaceae, and 22 in Russulaceae are highlighted by phylogenetical analysis. Some species have been identified with other species collected previously in other regions, while 28 others do not correspond to any species whose sequences are present in international databases and could be new species. In general, species harvested in Benin have more affinity with other African species. **Significance:** This study could be a beginning to invalidate the theory of "Global diversity and geography of soil fungi" since the sampling of ectomycorrhizal fungi within a single West African forest has shown a huge specific wealth not yet described. What about other African forests?

Barcoding in the deep sea: the exceptional diversity of giant protists (Foraminifera, Xenophyophora) on abyssal plains

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Background: Xenophyophores are giant agglutinated foraminifera (protists) and constitute a major part of the abyssal megafauna. They play an important role in deep-sea ecosystems, providing habitat structures for meio- and macrofaunal benthos and enriching the organic content of sediments surrounding their tests. This enigmatic group of protists has been recognized as a subclass of Rhizopodea, and the first molecular genetic studies conducted on this group have shown that they branch among single-chambered foraminifera. Two main groups, the stannomids and the psamminids, have been recognized based on morphological characters, but this division is not supported by recent molecular analyses. **Results:** We collected material from different sites of the Clarion-Clipperton Zone, a region in the eastern equatorial Pacific known for the presence of polymetallic nodules. We could identify 28 species of xenophyophores for which we obtained the barcoding fragment of the SSU rRNA gene. Of these, 26 species are new to science. Molecular data of four xenophyophoran species have been published previously; our work therefore results in a 7-fold increase of barcoded species for this group. **Significance:** Our study emphasizes the high diversity of xenophyophores and suggest that numerous unknown xenophyophore species remain yet to be discovered.

Application of DNA barcoding to identify the tropical timber trees from southern Yunnan

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Background: Illegal logging and trade with illegal timber and wood products are believed to be the main causes of worldwide deforestation and unstainable forestry. However, precise identification of timber and wood products is hampered by lack of standardized and validated forensic identification tools. DNA barcoding provides a promising forensic method for reliable timber identifications. **Results:** In this study, we evaluated four widely recommended plant DNA barcodes, rbcL, matK, trnH–psbA, and ITS2, for 212 individuals representing 103 tropical timber trees preserved in Xishuangbanna Tropical Botanical Garden in China, where are stored thousands of tropical plants all over the world. Our results showed that rbcL had the highest barcode recovery rates (95.28%), followed by matK (91.98%), trnH–psbA

(88.70%), and ITS2 (76.42%). Among the four fragments, ITS2 had the highest identification success rate (93.10%), followed by trnH–psbA (89.74%). The combination of matK + trnH–psbA + ITS2 provided the best discriminatory power (95.65%), followed by trnH–psbA + ITS2 (93.88%). Although the barcodes and their combinations showed high discrimination efficiency in most groups, it is hard to identify the timber trees from some complex groups, such as Dipterocarpaceae, Sapindaceae, and Sterculiaceae. Considering the overall performance of DNA barcodes, we suggest ITS2 and trnH–psbA as the more suitable barcodes to identify tropical timber trees. **Significance:** This study indicates that DNA barcoding would be an efficient forensic tool for timber identification to combat illegal logging and adulteration activities, especially in tropical areas with high biodiversity.

DNA barcodes and trajectories of body mass in Canadian Coleoptera

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Background: Body mass varies by over 16 orders of magnitude in the animal kingdom, variation with major impact on virtually all aspects of their morphology, life history, and ecology. Consequently, a deeper understanding of the patterns and covariates of mass variation is of key importance to understanding evolutionary trajectories. Coleoptera are an ideal taxon for investigation because their high species richness is coupled with marked size variation as well as occupancy of remarkably diverse and specialized niches. The Coleoptera fauna of Canada includes nearly 9000 species spanning over five orders of magnitude in mass. Most of these taxa have DNA barcode records, enabling analysis of how sequence data can reveal species relationships and inform evolutionary trajectories in body mass. **Results:** The body masses of over 3000 Canadian Coleoptera species, including representatives from over 100 families, were quantified. Feeding mode and habitat information of these species were obtained from the literature. COI sequences for these species coupled with a backbone family phylogeny were used to construct Bayesian and maximum likelihood trees. The resultant tree made it possible to investigate evolutionary trajectories in body size, enabling the evaluation of both phylogenetic constraints and the influence of ecological specialization. **Significance:** By documenting variation in the body mass of 1/3 of the Canadian Coleoptera fauna, this study reveals the importance of phylogenetic constraints and their interplay with ecology in evolutionary analyses. The new information on body mass and ecological traits also enriches data on Barcode of Life Data Systems (BOLD), a key step in extending its value for studies in contexts ranging from community ecology to improving the interpretation of metabarcoding studies by allowing mass adjustment of read counts. Additionally, the acquisition of similar information on body mass in other animal groups would substantially improve the parametrization of general ecosystem models.

An evolutionary odyssey to the Mediterranean Islands — the phylogeographic tale about insular freshwater amphipods

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Background: The Mediterranean Islands are considered as natural evolutionary laboratories, being among the most precious biodiversity hotspots in the world. Although the Mediterranean Region is housing about 6% of world freshwater taxa and its fresh waters are among the most endangered ecosystems, the knowledge about freshwater biota is very scarce, with very few studies focusing on the phylogeography of the insular fauna. Gammarid amphipods are the keystone species of the insular lotic macroinvertebrate communities; however, no biogeographical studies nor molecular bioassessments have yet been done on the insular freshwater gammarids. **Results:** The multi-marker dataset is composed of more than 600 individuals from nearly 100 sites from the Mediterranean Islands, including Sicily, Sardinia, Crete, the Aegean Islands, and Malta. The reconstruction of a time-calibrated phylogeny revealed the complexity of gammarid evolutionary histories, usually dating back to the major geological and isolation events of the particular island, also indicating that the colonisation of the insular inland waters took place either from the continental waters or from marine Tethyan waters. Moreover, all molecular operational taxonomic unit (MOTU) delimitation methods applied in our study support the extremely high level of overlooked diversity, ranging up to 15 distinct lineages within one morphospecies. **Significance:** Our results indicate the connection between the evolutionary history of the insular freshwater gammarids and the geological history of the Mediterranean Islands. The factual level of molecular diversity, largely exceeding the number of currently described gammarid species from the islands, combined with high local endemism, point out the importance of the DNA-based tools in freshwater biodiversity surveys. The results provide also a starting point for more studies on the ecological interactions and threat assessment for the newly discovered diversity. Given that freshwater ecosystems are under heavy anthropogenic pressure and their fauna is most prone to mass extinctions, there is an urgent need for further studies on the insular freshwater biodiversity.

Effects of PAH contaminants on meiofauna and microbial community structures in Baltic Sea sediments

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Background: Efforts to study the effects of chemical contaminants on the structure and function of microbial and meiofauna communities have traditionally focused on single contaminants and single species. This has left the complex interactions between mixtures of contaminants and their effects on the functions and structure of sediment microbial communities mostly overlooked. In our effort to improve our insights on these effects, we set up an experiment with the aim to study the interactions between a mixture of reported organic contaminants and microbenthic organisms. In this experiment, we spiked pristine Baltic Sea sediments with an ecologically relevant mixture of seven organic contaminants. We then used metabarcoding to monitor changes in microbial and meiofauna diversity and structure in response to the contaminant mixture. In addition, following previous data on the exposure of contaminants on benthic microbial activity, we investigated key genes in the microbial nitrification and polycyclic aromatic hydrocarbon (PAH) degradation pathways. **Results:** In our preliminary results, we found a notable difference in meiofauna community structure in contaminated sediments. Amongst microbial communities, the abundance of active PAH degraders was found notably higher in contaminated sediments, as they were below the detection limit in untreated samples. The quantification of AmoA cDNA copies varied widely between timepoints compared to untreated sediments. We are currently still processing sequencing data for bacterial diversity and community structures. **Significance:** Benthic communities play a fundamental role in regulating important ecosystem functions. Currently, how benthic interactions and biodiversity are affected by contaminant exposure is not well understood. Our study shows that mixtures of complex organic contaminants significantly change microbial community structure with potential concomitant functional effects. These effects are important to consider in predicting and assessing the risks of contaminant mixtures at an ecosystem level.

DNA barcoding analysis and phylogenetic relationships of associated species of *Juniperus polycarpos* in Juniper forest Ziarat, Balochistan

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Background: DNA barcoding, a recent revolution in the field of molecular genetics, is gaining popularity due to its quick and accurate identification of plants, contrary to subjective biases associated with morphology-based identification of taxa. The core plant barcode, rbcL+matK recommended by the Consortium for the Barcode of Life (CBOL) Plant Working Group, needs to be evaluated for a wide range of forest plant species. **Results:** Here we developed DNA-based plant biodiversity inventories and tested the potential of the rbcL+matK marker for the identification of associated flora belonging to diverse families in Juniper Forest ecosystem of Ziarat, Pakistan. Maximum likelihood and neighbour-joining tree analysis were performed to evaluate the discriminatory power of the rbcL+matK gene. Our findings showed that amplification success of rbcL is 98% as compared to 76% for matK. The sequencing success of rbcL is better as compare to matK. A total of 320 DNA barcodes representing more than 100 plant species of 50 families were successfully generated. RbcL gene sequences enabled identification of the majority of the samples (75%) to genus level and only 25% to species level, while matK identified 72% to genus level and only 28% to species level. Some of the species are not in the NCBI and BOLD databases, so the marker showed resolution at family level. This is because most of these species are endemic to Juniper flora of Ziarat. It is concluded that rbcL has good amplification and nice resolution at genus level, while matK showed fair amplification and sequencing but nice resolution at species level. **Significance:** This study enables us to explore the unhidden parts of Ziarat Juniper valley, as new plant data are in connection with the whole world, which helps us to explore in diversified field of study.

Writing the Encyclopedia of Life: DNA barcoding of Finnish spiders in context of intra- and intercontinental comparison

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Background: Spiders remain a relatively poorly studied group of animals. It is estimated that up to 130 000 species are awaiting discovery. DNA barcoding offers the unprecedented opportunity to reveal new and overlooked species, to estimate geographical variation, and to standardize taxonomic routine. Arachnologists greatly benefit from available public DNA barcode datasets covering whole countries and problematic species groups, but there is still a great need for comprehensive datasets covering large geographic areas. **Results:** We assembled a DNA barcode library for Finnish spiders that consists of 1741 sequences belonging to 372 species, 184 genera, and 28 families. The library covers 57% of the species, 70% of the genera, and 88% of the families known for Finland. We observed 260 concordant BINs (including splits), 11 discordant BINs (cases of barcode sharing), and 107 singletons. Preliminary comparison of Finnish records to publicly available DNA barcode data revealed deep interspecific splits (>2%) and a high proportion of haplotype sharing cases based on BIN analysis (25% and 16% correspondingly). These values are higher than reported previously at the level of a single country. At the same time, the percentage of such cases detected within Finland is similar to Canadian and German datasets (7% of splits and 6.5% of sharing). Given that the European fauna is relatively well studied, many of these cases likely indicate complex biological processes rather than misidentifications at a local scale. However, complex cases detected by comparison of our dataset to Canadian and other distant countries' records could possibly correspond to incorrect taxonomy, recent species expansion, and adaptive variation. **Significance:** We investigate problematic cases and present preliminary conclusions regarding the observed patterns. The DNA barcode library for Finnish spiders contributes to a better understanding of evolutionary processes within arachnids and supports the Encyclopedia of Life written by the global scientific community.

Coupling DNA barcoding with NGS assessment of cyanobacterial and algal community composition in harmful blooms

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Background: Our ability to monitor and remediate impaired waterbodies is often severely challenged by their vast geographical scale and distribution. Cyanobacterial morphology is affected by environmental conditions, resulting in identification discrepancies between different labs. Here, we are applying DNA barcoding and Next-Generation Sequencing (NGS) to develop a rapid assessment toolset for two important indicators of ecosystem health: toxigenic bloom-forming cyanobacteria and impaired planktonic biodiversity. The project was designed to accomplish three major goals: (1) build a reference BOLD database for algal and cyanobacterial 16S ribosomal RNA, using isolates from the Great Lakes and other regions; (2) optimize PCR amplification and DNA extraction methods using mock communities; and (3) analyze environmental samples using targeted NGS with specific 16S primers to simultaneously detect cyanobacteria and eukaryotic algal chloroplasts. **Results:** Up to date reference database contains 203 cyanobacterial and algal strains and 101 species, with focus on blooming and toxin-producing taxa. Using reference database sequence data and mock communities for protocols validation, we developed a new NGS primer set, which improved recovery of the following eukaryotic orders: Zygnematales, Volvocales, Ochromonadales, and Euglenales. We also developed cost-efficient DNA extraction protocols matching performance of commercial kits. Our bioinformatics pipeline was designed to handle low taxonomic resolution for problematic genera of cyanobacteria. Samples collected at four stations in Lake Erie in August 2015 and May 2016 were analyzed using microscopy and NGS. Cyanobacteria were dominant in samples from August 2015 based on cell count and NGS. With an exception for one station, more genera were detected using NGS. **Significance:** Optimized NGS protocols, a customized bioinformatics pipeline, and the BOLD 16S reference database coupled with the Ion Torrent S5 instrument enable rapid assessment of cyanobacterial and algal community composition in harmful blooms. The resulting pipeline was used for processing of 124 environmental samples.

Integrative taxonomical methods in revealing cryptic diversity of freshwater *Palaemon* shrimps

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Background: The caridean genus *Palaemon* (Palaemonidae, Decapoda) is very diverse, speciose, and distributed worldwide, including marine, brackish, and freshwater taxa. Until now, seven species were described from fresh waters of Europe. Among them, *Palaemon antennarius* has the widest distribution and type locality in the Lake Trasimeno, Apennine Peninsula. **Results:** We investigated *Palaemon*

shrimps occurring in the Lake Skadar, the largest water body in the Balkan Peninsula, very shallow and formed only ca. 1200 years ago, as a result of the overflowing of an old polje – marsh area rich in karst springs. The morphology and molecular diversity (mtDNA COI) of the local shrimp population, so far considered as *Palaemon antennarius*, was compared to conspecifics collected over the entire Apennine Peninsula and to *Palaemon minos*, endemic to Lake Kourna, Crete. Molecular analysis revealed high genetic diversity in the studied dataset. Shrimp collected on the Apennine Peninsula formed two clearly separated clades. *Palaemon* from Lake Skadar differed significantly from the Italian population, being presumably a new yet unknown species, most probably endemic to the Lake Skadar basin. Geometric morphometry methods based on the shape relations in carapace and rostrum followed by procrustes analyses confirmed distinctness of the Skadar population from both the Apennine *P. antennarius* and the Cretan *P. minos*. Surprisingly, based on the preliminary time-calibrated reconstruction of phylogeny, the Skadar population turned out to be a sister clade of *P. minos*, from which it diverged ca. 1.2 Ma. However, a multimarker dataset is needed to clarify this situation. **Significance:** Our results show high potential of DNA barcoding combined with advanced morphological studies for detection of cryptic and pseudo-cryptic diversity. It underlines also the need for taxonomic studies upon the diversity of freshwater taxa in the Mediterranean biodiversity hotspots, even in the case of presumably well-known groups such as the decapods.

Evaluation of Arabian plant barcodes: an effort on integration of machine learning approach towards accurate species identification

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Background: Arabia is the largest peninsula in the world, with >3000 species of vascular plants. However, not much effort has been made to digitally catalogue these plant species. Integration of species taxonomy with genetic data could assist in accurate identification to maintain the genetic identity and integrity of species in their natural habitat. The accuracy of species identification relies on the degree of congruence between the DNA barcodes and the species taxonomy of the group being assessed. It was effective for animals using partial cytochrome oxidase I (COI) mitochondrial gene sequences. But for plants, it has been more challenging, as the rate of species discrimination for the core barcode markers rbcL and matK (CBOL) is not more than 70% (CBOL plant working group). Even by using different methodological strategies, plant identification is prone to be confounded by the absence of a barcode gap. The accuracy of species identification not only relies on the selection of suitable markers, but also on the efficiency of the computational method that could surge the rate of classification. The aim of our study was to evaluate rbcL and matK barcodes deposited in a decade from Arabia (9 countries) at NCBI GenBank using genetic distance-based method for Aligned (AL) datasets and character (K-mer) based Supervised Machine Learning Algorithms (SMLA) for Aligned (AL) and Alignment-Free (AF) datasets. **Results:** This study represents the first comprehensive assessment of plants from the Arabian Peninsula emphasizing efficiency of genetic distance and character-based Supervised Machine Learning methods for DNA barcode analysis. We analyzed 1341 DNA sequences of Arabian plants belonging to the rbcL and matK markers. The sequences were filtered and curated to obtain 943 rbcL barcodes belonging to 377 species and 243 matK barcodes for 84 species. The analysis progressed through AL set of sequences and AF set of sequences with K-mer frequencies achieved through Logical Alignment Free (LAF) algorithm. The genetic distance-based method (TaxonDNA) was able to successfully discriminate 127 species belonging to rbcL and 3 species to matK. For character-based identification, four classifiers were employed (Decision tree, k-nearest neighbor (kNN), NaiveBayes, Sequential minimal optimization (SMO)). These classifiers were implemented through an informatics workflow to surge classification accuracy. The SMO classifier exhibited true positive rate (TPR) for highest number of species of 177 for AL dataset, while kNN was able to accurately resolve 149 species from AF dataset belonging to the rbcL. For matK, SMO and K-NN classifiers showed the highest TPR of 13 for aligned and 11 for alignment-free datasets respectively. **Significance:** Our attempt successfully demonstrates the efficiency of the genetic distance and character-based SMLA in species discrimination, where SMLA scored the highest rate in taxonomic discrimination, suggesting the necessity of these classifiers in evaluation of plant taxa. Moreover, the curated DNA barcodes could assist in the form of a reference library to improve DNA barcode identification success rate for Arabian plants in a more extensive way.

Ten years of DNA barcoding in diverse educational contexts: lessons learned and future potential

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Background: DNA barcoding is intuitive and simple compared with other techniques in molecular biology, and its applications are compelling and practical. For these reasons and others, DNA barcoding, and now metabarcoding, have undeniable appeal for hands-on STEM education and learning. As more and more DNA barcoding practitioners and educators implement the technique in formal and informal educational settings, the field of DNA barcoding is simultaneously growing and evolving. What have we learned? How can new developments, particularly metabarcoding and next-generation sequencing, be incorporated? How can we harness the educational potential of DNA barcoding? **Results:** In this talk I will share my experiences from 10 years of implementing DNA barcoding and metabarcoding in diverse educational contexts including a residential program for 11–14-year-olds, short courses for undergraduates and their professors, summer research opportunities for high-school and college students, and participatory (citizen) science programs for adults. I will condense these experiences into lessons learned, supported with data and anecdotes from student and participant evaluations, as well as project evaluations. I will describe the conceptual and practical challenges of implementing next-generation sequencing in educational settings, describe how I have incorporated Oxford Nanopore Technologies' MinION sequencing device into my own teaching and training, and report on the results of a survey of 100 educators on if and how they would use the MinION in their own teaching. **Significance:** I will discuss the future potential of DNA barcoding and metabarcoding in education, and map out how we get there from here. I will amplify the call for the development of a community of practice around DNA barcoding and metabarcoding in formal and informal education. Finally, I will make recommendations for how the DNA barcoding community can both support and benefit from educational applications of barcoding and metabarcoding, including the development or improvement of methods, technologies, analytical tools, and training opportunities.

BioAlfa — to DNA barcode an entire tropical country for the survival of its biodiversity

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Background: Costa Rica still has at least 1 000 000+ multicellular species living wild in the variously conserved terrestrial and wildland 25% of the country. For these to survive through their long-term acceptance by Costa Rica's 5 million national inhabitants, we need to be coming to know who they are, where they are, what they do, and how to find them – and get their information available to the internetted public. With a 10-year effort of citizen-sourcing and nation-sourcing, it is now feasible to actually do this by combining national sweat-equity

and human resources, international collaborations, and the technology in hand. **Results:** Costa Rica is starting to DNA barcode its entire country, and facilitate the use of the results for biodiversity conservation through its use in many different kinds of biodevelopment. This project is termed BioAlfa for its effect of rendering a country bioliterate (which is bioalfabetizado in Spanish). The pilot project is Area de Conservacion Guanacaste (ACG), a 169 000 ha conserved wildland in northwestern Costa Rica (www.acguanacaste.ac.cr), that has invested 37 years and more than $107 million demonstrating that it is possible to integrate a wildland with its society. A few examples will be presented. **Significance:** The BioAlfa attitude and realities will spread widely in the tropics. This will support tropical biodiversity conservation in larger areas and other countries, demonstrating that DNA barcoding is more than just a fascinating technical breakthrough for applied science. Loss of wild biodiversity is widely lamented in the extratropical regions of the planet, and by the residents of those societies. However, its much greater loss in the vastly more biodiverse tropics is lamented by few people and entities. Costa Rica is attempting to address this imbalance through conducting a DNA barcode-based inventory and wild organism biodevelopment for its own conservation and benefit, in order to produce a bioliterate tropical country.

First new alien freshwater species in Georgia identified by DNA barcoding

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Background: Georgia is considered a distinguished biodiversity region, as it contains two biodiversity hotspots: the Caucasus and the Iran–Anatolian hotspots. However, our understanding of Georgia's biodiversity, especially invertebrate animals and freshwater realm as a whole, is far from complete. Species identification by DNA barcoding may represent an alternative to traditional methods of species identification, and at the same time provide useful information on phylogenetic positions and cryptic lineages. We used DNA barcoding to check species identity in gobies and crabs of Georgia. **Results:** Fish and crabs were collected from the freshwaters of West and East Georgia. A rapid and preliminary identification of collected fish was done using traditional taxonomic methods followed by DNA isolation and COI amplification with universal primers. Four species of gobies in three genera and two species of crabs belonging to two genera were identified. Among them were two invasive species, *Rhinogobius* cf. *brunneus* and *Rhithropanopeus harrisii* detected for the first time in Georgia. **Significance:** An increasing number of species is being introduced into new environments either by accident or deliberately. In order to protect native biodiversity, early detection and management of established alien species populations is important. Our results showed that molecular methods can be used for rapid species identification and effective detection of new introductions. The results encourage us to increase the taxon sampling in a wider geographical area in order to develop a comprehensive database of the freshwater fauna of Georgia.

Investigating the potential of using eDNA from water samples for detecting stream-associated arthropod communities in Denmark

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Background: Environmental DNA (eDNA) has recently emerged as a promising tool for species detection on broad taxonomic scales. While the method has received a lot of attention in tracing vertebrate communities, invertebrate communities have been less studied, partly due to their high diversity and lack of genetic resources in reference databases. Insect orders such as Ephemeroptera, Plecoptera, and Trichoptera are widely used as bio-indicators of water pollution, but monitoring with traditional methods is time demanding and, therefore, costly. Using filtered water samples from five streams located in the vicinity of Aarhus, Denmark, we here explore the efficacy and precision of using metabarcoding in detecting arthropod community compositions. **Results:** Samples were collected in early summer and late autumn to account for different life stages and to explore how seasonality in arthropod community composition is expressed in eDNA sequencing data. Using a near-decadal dataset of arthropod species occurrences and abundances compiled by the national monitoring program in Denmark, NOVANA, from the same five locations, we compare our findings and evaluate the methodology in a broader context. As genetic reference databases are compiling, eDNA methods are becoming increasingly useful for species detection of highly diverse taxa. **Significance:** Understanding how eDNA methods could supplement traditional observational methods is crucial, and this study highlights the applicability of utilizing eDNA methods in arthropod species detection in aquatic environments.

SPIKEPIPE: a metagenomic pipeline for the accurate quantification of eukaryotic species occurrences and abundances using DNA barcodes or mitogenomes

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Background: The accurate quantification of eukaryotic species abundances from bulk samples remains a key challenge for community description and environmental biomonitoring. **Results:** We resolve this challenge by combining shotgun sequencing, mapping to reference DNA barcodes or to mitogenomes, and three statistical filters: (1) a percent-coverage threshold to filter out false positives, (2) an internal-standard DNA spike-in to correct for stochasticity during sequencing, and (3) technical replicates to correct for stochasticity across sequencing runs. This pipeline achieves a strikingly high accuracy of intraspecific abundance estimates from samples of known composition (mapping to barcodes $R^2 = 0.95$, mitogenomes $R^2 = 0.93$) and a high repeatability across environmental-sample replicates (barcodes $R^2 > 0.95$, mitogenomes $R^2 > 0.95$). **Significance:** As proof of concept, we sequence arthropod samples from the High Arctic systematically collected over 17 years, detecting changes in species richness, abundance, and phenology using either barcodes or mitogenomes. SPIKEPIPE provides cost-efficient and reliable quantification of eukaryotic communities, with direct application to environmental biomonitoring.

Mitobarcoding: a method for the detection of biological ingredients from mixtures based on whole genome sequencing

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Background: The metabarcoding method has been widely used to identify the source materials from biological mixtures. However, the standard procedure uses only one marker and needs PCR amplification, which has several limitations. Here, we aim to develop a method that is able to identify biological ingredients from multiple unknown

mixtures without the limitation of using only one marker and PCR amplification. **Results:** We used Next-Generation DNA Sequencing (NGS) technology to directly amplify the DNA extracted from a biological mixture. A computational pipeline (called bio-ingredient identification based on mitochondrial reference genome sequences, or BIID-mito) was developed to qualitatively and quantitatively identify the biological ingredients from the mixture. The BIID-mito software package includes a database and two programs. The database consists of the mitochondrial genomes. The first program generates simulated fastq sequences. The second one maps the NGS reads to the reference sequences and counts the numbers of reads, which is used to qualitatively and quantitatively determine the biological ingredients in the mixture. We use a mixture constructed with samples from 18 animal species as a testing sample. BIID can successfully identify 16 of 18 added species. The identification results were confirmed with Loop-mediated isothermal amplification (LAMP) experiment. We then compared the mitobarcoding results with those of several commonly used markers, such as COX1, 12S rRNA, and 16S rRNA. The mitobarcoding showed better sensitivity and specificity. Lastly, we constructed samples containing two ingredients at various proportions. The metabarcoding method was applied and identified the ingredients quantitatively. **Significance:** The mitobarcoding method has two advantages. First, it uses mitochondrial genomes as a superbarcode rather than a single marker. It does not depend on universal PCR primer pairs to amplify particular markers. Second, it is possible to determine the contents of a biological mixture quantitatively.

Development of species identification marker for *Anas poecilorhyncha* and *Anas platyrhynchos*

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Background: Most aves can be identified using the mitochondrial COI gene, whereas *Anas platyrhynchos* and *Anas poecilorhyncha*, avian influenza-susceptible birds, are not distinguished by the COI gene. Both *Anas* species have importance in the field of avian influenza research and ecological research of birds. **Results:** A species identification marker for the two species was developed based on whole genomes produced by next-generation sequencing. To develop the marker, we explored indel regions of the genomes and used 15 individuals of *A. poecilorhyncha*, 10 individuals of *A. platyrhynchos*, and a hybrid individual of the two species. Finally, we confirmed that the two species were distinguished by one indel region among seven candidate markers, and that the indel marker possibly enables detection of hybrid individuals. In the sequence alignment, the indel region of 49 bp length showed up in *A. platyrhynchos*, but not in *A. poecilorhyncha*. **Significance:** It is expected that the newly developed marker can be used for avian influenza surveys and ecological studies.

The COI barcode database of Brachyura and Anomura collected along the Korean Peninsula over five years

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Background: Many taxa belonging to Brachyura and Anomura (crabs and allies) play a key intermediate role in the maintenance of coastal ecosystems. The diversity information of these taxa can be used to create a bioindicator protocol that provides insights into contemporary food web complexity and stability. The barcoding genetic markers for Brachyura and Anomura on the Korean Peninsula, where there are extremely diverse ecosystems with complicated coastal topology and current structure, have not yet been properly developed. **Results:** Over the past five years of investigation, 122 species of 37 families of Brachyura and Anomura have been collected, and a cytochrome c oxidase I (COI; 658 bp) DNA barcode database was constructed to identify variabilities within and between those species. The COI gene used showed a relatively conservative pattern at the species, genus, and family levels with only a few exceptions. Character-based barcodes could thus be generated in more than 90% of the species used. **Significance:** Our phylogenetic analysis detected several species with extensive variability interfering with the monophyly formation, suggesting the existence of cryptic species complex. Some taxa belonging to the same genera or families were often found to be separated into clearly different clusters, requiring taxonomic revisions. Our genetic data are the most important progress in establishing a management barcode system for coastal creatures and can also greatly contribute to the control of fisheries around the Korean Peninsula.

Combined multivariate analysis and DNA barcoding studies unravel the species complexity in *Ficus* (Moraceae) endemic to South India

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Background: *Ficus*, a complex genus currently circumscribed with ~735 species under six subgenera, is distributed all over the world. *Ficus* species are very variable in morphological characters and exhibit a wide range of often partially overlapping distributions, which makes the identification very difficult. The systematic classification within and between the subsections of *Ficus* is problematic; e.g., it is still unclear where to classify *F. benghalensis, F. krishnae, F. middletonni, F. amplocarpa, F. amplissima*, and so on. In this study, we analysed the morphological variations among southern Indian *Ficus* species using a numerical taxonomic approach based on overall similarity and DNA markers, to investigate the intraspecific and infraspecific variations in circumscribed groups. Based on 141 OPU and 55 morphological characters, multivariate analyses were performed. To clarify the circumscription of *Ficus*, a phylogenetic reconstruction based on ITS2 and trnH–psbA combined with morphology were carried out. **Results:** This combined study clearly proves that there is a need for a new classification of *Ficus* species based on morphological and DNA markers. The multivariate analyses showed new associations among species and genera, which were partially in agreement with previous classifications, corroborating the importance of phenetic analyses in evaluations of taxonomic entities. DNA markers (ITS2 and trnH–psbA) also showed variations between the species, e.g., *Ficus amplissima*, formerly constituting a sect. *Leucogyne* appear to be embedded in subsection *Conosycea*. Results of phylogenetic analysis necessitate nomenclatural adjustments. **Significance:** This is the first effort to compile both morphological traits and DNA markers to restructure a new classification of *Ficus* species.

Utility of eDNA and eRNA for monitoring freshwater zooplankton diversity under acidification stress

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Background: The use of environmental DNA (eDNA) in aquatic biodiversity surveys is gaining considerable interest, particularly with global observed declines in freshwater biodiversity and ecosystem health. However, due to the complex spatio-temporal dynamics of eDNA potentially limiting its applicability in determining current, viable species assemblages, the use of environmental RNA (eRNA; RNA collected from the environment in the absence of the progenitor organism) has recently been proposed as a potential alternative for biodiversity surveys due to its comparatively rapid degradation rate. However, the ecology of eRNA in aquatic ecosystems remains largely

unknown. Our research aims to validate the utility of eRNA as a more suitable alternative to eDNA in detecting zooplankton biodiversity turnover over relatively short time periods. We use a large-scale mesocosm experiment, involving an array of 32 ~1000 L artificial ponds exposed to various degrees of acidification stress over a 12-week period. We predict that eDNA signals will persist in the environment for a longer time span than eRNA due to slower degradation rates, thus obscuring the detectability of species turnover. Consequently, eRNA may serve as a more accurate indicator of current, viable species assemblages. **Results:** We find that eRNA-based species detection demonstrates a better correlation with morphological samples and zooplankton species turnover in comparison to eDNA. While eDNA metabarcoding recovered twice as many species as eRNA, many such detections are likely false positives (terrestrial species such as insects, fungi, etc.), or residual signals from past communities. **Significance:** As the potential for utilizing eDNA and eRNA metabarcoding for biomonitoring increases, it is important to verify the effectiveness and utility of both methodologies in quantifying aquatic biodiversity. Overall, this work contributes to the validation of eRNA as a biomonitoring tool in tandem with eDNA, and may be utilized to inform future biomonitoring strategies.

Unveiling the rich Trichoptera diversity in Bosnia and Herzegovina — the case of *Rhyacophila bosnica*

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Background: Rich geomorphic and hydrographic features of Bosnia and Herzegovina conditioned the development of a diverse and abundant fauna of Trichoptera, characterized by a high level of endemism. The freshwater fauna of caddisflies from the Balkan Peninsula and Bosnia and Herzegovina are underinvestigated, with many species still missing the morphological description of developmental life stages and(or) DNA barcode data. Such a species is *Rhyacophila bosnica* Schmid, 1970, endemic to the Balkan Dinaric region, with unknown morphology of its larva and no DNA barcode sequence. This study aimed to provide the first DNA barcode sequence for endemic species *R. bosnica* and to utilize it to link adult and larval life stages. **Results:** The analysis of the COI region in adult *R. bosnica* specimens resulted in the entire barcode sequence of 658 bp, while for larva 341 bp of the second half of COI barcode was retrieved using mini barcodes. A search of Barcode of Life Data Systems (BOLD) showed genetic distance of over 10% for both samples, which indicates that there are no records of the investigated species in BOLD yet. **Significance:** In BOLD, there are only 196 DNA barcode records with species-level information out of 700 known species from the genus *Rhyacophila*. The barcode data for a third of the *Rhyacophila* species recorded in the Federation of Bosnia and Herzegovina are still missing from BOLD. MSA analysis of the consensus sequence from this study and those of *Rhyacophila* species in GenBank revealed that more than 45% of sites are polymorphic among different species of *Rhyacophila*. This finding corroborated that sequences obtained from the specimens in this study indeed belong to the same species, enabling us to link two different life stages of *R. bosnica* using DNA barcoding approach. The positive pairing sets a reliable basis for subsequent morphological description of *R. bosnica* larva.

DNA barcoding reveals unexpected diversity of the pink-coloured *Clavaria* (Basidiomycota, Clavariaceae) species of the *Clavaria incarnata* s.l. group

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Background: The genus *Clavaria* (Basidiomycota, Clavariaceae) is characterized by small, simple, club-shaped or cylindrical basidiomata, mostly growing in semi-natural grasslands and shrubs. Because of simple structure and lack of taxonomical characters, the real diversity of the genus remained unknown until the discovery of molecular taxonomic techniques. *Clavaria incarnata*, described in 1910, is a rarely recorded, pink-coloured species of the subgenus *Holocoryne* characterized by loop-like clamps at the base of the basidia. Diversity of the spore size and ornamentation, as well as presence/absence of cystidia in the hymenium, led to the assumption that *C. incarnata* is in fact a species complex. However, this has been for a long time unsolvable by the means of classical taxonomy. In 2015, based on the results of DNA analyses, the first species, *Clavaria mesapica*, with conspicuous cystidia in hymenium, has been separated from the group. In 2019, *Clavaria microspora* species with the smallest spores within the group followed. **Results:** Recently, large-scale DNA barcoding and phylogenetic analysis of more than 100 *Clavaria* specimens revealed at least four other separate clades, representing separate species differing also in spore size and ornamentation. Phylogenetic relationships were inferred using nuclear ribosomal internal transcribed spacer and partial large subunit (ITS, LSU rDNA). The analyses recovered a clade, which is probably conspecific with the type species *Clavaria incarnata* s.s. Taxonomy and description of the remaining three species is also presented.

Automated reference database creation for any marker and taxonomic group

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Background: An up-to-date and high-quality reference database is essential for DNA barcoding and metabarcoding. Given the variety of markers, formatting requirements for classifiers, and constant growth of primary databases, suitable reference databases are, however, limited to few markers. Other common problems are lack of standardization or documentation, and outdated data. **Results:** We developed a software pipeline with a web and command line interface to generate reference databases on-the-fly for any applicable marker. It gathers current available data from primary databases and allows for optional filtering, formatting, and restriction options specific for (meta)barcoding purposes. Generated databases optionally receive a DOI, making them well documented with meta-data, publicly sharable, and citable. **Significance:** BCdatabaser enables researchers to quickly build standardized reference databases for arbitrary markers and custom taxonomic groups. It helps capitalize on new data while maintaining quality and reproducibility. Availability: https://www.github.com/molbiodiv/bcdatabaser (code), https://bcdatabaser.molecular.eco (webservice).

(Meta)barcoding plant–pollinator networks in agricultural habitats

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Background: Pollination is crucial for maintaining angiosperm biodiversity and represents one of the most important ecosystem services. With the increasing threats of massive insect decline, studying pollination and associated networks has become more important than ever. However, studying plant–pollinator interactions at a species level with morphological methodologies is time-consuming, expensive, and depends on exceptional taxonomic expertise. In this study, we target the plant–pollinator networks of two important crops

(caraway and apple) using a combination of traditional methods with DNA barcoding and metabarcoding. With this approach, we can identify potential dipteran and hymenopteran pollinators and from their pollen loads their associated plant species. **Results:** In total, during three vegetation periods in apple orchards and two in caraway fields, more than 5000 insect specimens (Diptera: Brachycera, and Hymenoptera) have been collected individually with a standardized approach using hand-netting. In the caraway fields, 84 brachyceran and 49 hymenopteran potential pollinator species have been identified. Insect samples from apple are still under study. Morphological identification of pollen samples from the first 555 potential pollinator specimens yielded 90 different plant taxa. Metabarcoding of pollen samples is still in progress and will extend and verify the results of the morphological identification. The number of different insect and plant species indicate strikingly complex plant–pollinator networks in our two target crops. **Significance:** This study is one of the first assessing the diversity of potential pollinators and the structure of plant–pollinator networks in agricultural habitats with an integrative approach including (meta)barcoding and explicitly targeting understudied potential pollinators such as true flies and nonbee hymenopterans. Results will help advising farmers on understanding and promoting pollination diversity, and more generally biodiversity in agricultural habitats. Targeted wild pollinator management as in FAP (Farming with Alternative Pollinators) will be easier and help to reduce pollination deficits and enlarge crop safety, quality, and yields.

DNA barcoding of leafminer larvae pressed within leaves of herbaria shed light on its invasion history

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Background: Historical herbaria can significantly improve our knowledge about past ranges of endophagous arthropod pests. In the last few decades, the lime leafminer, *Phyllonorycter issikii* (Kumata, 1963) (Lepidoptera: Gracillariidae), has expanded over most of the Palearctic and became a pest of lime trees, *Tilia* (Malvaceae). Our recent phylogeographic analysis revealed unexpectedly high *Ph. issikii* genetic diversity in Europe (invaded region) versus East Asia (putative native range), questioning the hypotheses about its expansion and the region of origin. We examined the world's biggest herbarium collections containing *Tilia* specimens to find early evidence of *Ph. isikii* presence in Europe and in East Asia, and clarify its invasion history. **Results:** In the framework of a research initiative supported by the COST Action "Global Warning" FP1401, Le Studium and the Russian Foundation for Basic Research (No. 19-04-01029 A), we examined about 10 000 herbarium sheets containing ~1.5 million *Tilia* leaves sampled in the Palearctic between the end of XVIII century and the beginning of XXI century for presence of the typical *Phyllonorycter* mines. Overall, 103 sheets carried leaf mines, in some cases with larvae and(or) pupae inside, dated from the year 2014 back to 1859. We extracted DNA from 53 larvae and pupae and sequenced them using minibarcodes. Our preliminary results confirm the hypothesis of the recent occurrence of *Tilia*-feeding *Phyllonorycter* in the West and its long-term presence in the East. Data stored in archival herbaria shows several increases of population densities of the lime leaf miner in East Asia, way before the insect was formally described. Historical herbaria indicate that *Tilia*-feeding *Phyllonorycter* exists in China, which is a new record for this country. **Significance:** This study highlights the importance of historical herbaria and DNA barcoding to explore invasion histories of phytophagous arthropod pests.

Surveying insect community patterns along a forest conversion gradient from Norway spruce to European beech using eDNA from soil and Malaise traps

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Background: Forests are dynamic entities, characterized by complex reciprocal interactions between and within the abiotic and biotic environment. Forest fauna and flora are closely linked and are constantly interrelating and mutually influencing each other. The presented study investigates changes in insect community patterns along a forest conversion gradient from non-native Norway spruce (*Picea abies*) to European beech (*Fagus sylvatica*). The major aim of the study is to determine the progress of ongoing renaturation measures through underplantation in the Eifel National Park, Germany, by monitoring changes in species composition. **Results:** To capture a picture of the biological heterogeneity as completely as possible, flying arthropods were caught with Malaise traps while ground-dwelling organisms were assessed by extracting environmental DNA (eDNA) from soil samples. Extraction of eDNA from the Malaise traps was performed with a lysis buffer followed by salt precipitation. In order to allow for a better taxonomic resolution and a wider taxonomic coverage, we analysed two genes: the mitochondrial COI barcode region and the nuclear 18S rRNA gene. During the biodiversity assessment, we lay a special focus on the highly diverse insect order Diptera. Dipterans play a major role in forest health as they occupy almost all available ecological niches and serve inter alia as pollinators, biocontrol agents of insect pests, and decomposers of organic material. **Significance:** Our study clearly shows that eDNA metabarcoding is an accurate, time and cost-efficient alternative to surrogate measures of biodiversity. Moreover, it highlights the complex structure and dynamics of forest communities and points out the advantages of using total biodiversity on the basis of eDNA over indicator species for surveying changes in biological communities. More specifically, the Eifel National Park environmental forest gradient could serve as a proof of concept to identify possible interactions between forest mixing effects, soil composition, and insect biodiversity patterns.

Censusing coral reefs in the 21st century

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We still lack a well-constrained estimate for the diversity of marine life. The challenge stems from the fact that most marine species are small, rare, and undescribed by science. The challenge is particularly great for coral reefs, which are highly threatened, difficult to sample, and likely to host one quarter to one third of all marine species. For example, early studies of crustaceans associated with reef frameworks undertaken as part of the Census of Marine Life indicated that a few square metres of reef hosted nearly as many species as all European seas. Fortunately, high-throughput DNA sequencing approaches (meta-barcoding) combined with standardized environmental samples provide a cost-effective way to estimate the number of species in a specific location and compare such estimates with those made elsewhere. Autonomous Reef Monitoring Structures (ARMS) mimic the reef matrix by providing spaces in which small invertebrates and fish can shelter and surfaces onto which sessile organisms can attach. Ongoing analyses include samples from shallow-water reefs of the Caribbean and the Red Sea, mesophotic reefs of Curaçao, and reefs of Papua New Guinea adjacent to carbon dioxide seeps; the latter provide a window on the future impacts of ocean acidification. Genetic analyses reveal hundreds of species associated with each unit, with the smallest organisms being the most diverse, and rarefaction curves suggest that many more remain to be detected. Remarkably, the majority of these sequences cannot be matched to named species, and a surprisingly large fraction cannot be assigned to phylum, reflecting the fact that most marine life remains unrepresented in genetic databases.

Analyzing historical plant–pollinator interactions by conducting pollen metabarcoding on natural history collections of German bumblebee species

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Background: Bumblebees are considered keystone species in ecosystems, and declines in their abundance and diversity negatively affects other wildlife. Nineteen (19) of the 38 bumblebee species that occur in Germany are classified as rare or extremely rare. Very few studies have addressed the question of whether bumblebee declines are associated with loss of floral resources due to contemporary anthropogenic changes in landscapes. The advent of metabarcoding has provided new tools with the potential to investigate dependencies between floral resources and pollinator diversity in greater detail than was possible in the past. **Results:** Ongoing in-silico evaluation of newly generated primers aims at a reduction of mismatches across the European flora compared to currently available primers. Sequences generated from biomass (pollen, plant tissue, fungal spores, and other unknown components) adhering to the bumblebee's body potentially provides information on environmental interactions that were not observed in the analysis of the pollen provisioned for larval nutrition. **Significance:** Our effort to analyze pollen from natural history collections from three time periods of important agricultural shifts (before 1950, 1950–1980, after 1980) has the potential to provide valuable insight into interactions between bumblebees and the environment, possibly explaining bumblebee declines in Germany. Our sampling effort will be comprehensive, comprising 20 specimens per species and time period (~2250 specimens in total).

Measuring quantitative preferences to diet species by comparing gut contents and environments in numbers of DNA sequence reads from metabarcoding

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Background: While gut content analysis by metabarcoding may detect more diet species than morphological observation, challenges remain in quantifying their abundance. The numbers of sequence reads potentially reflect their relative volumes or cell frequencies in the gut content. However, they must be biased by variations among species in PCR amplification and in other processes. In this study, we focused on pond smelt, *Hypomesus nipponensis*, which is an important fishery species in Japan, and calculated smelts' average functional responses to their diet species. To obtain the numbers of sequence reads for the diet species (or genus) in smelts' guts and the environment, DNA extracted from gut contents and bulk DNA of zooplankton collected by using a plankton net in the lake were subjected to metabarcoding analyses of COI. The collected zooplanktons were also identified morphologically, to examine whether the number of sequence reads can be used as quantitative measures for abundance. We then calculated the type-I functional response to each diet species, i.e., the ratio of [read number in gut] to [read number in environment], to get a rough but relatively unbiased quantitative preference by the fish species. **Results:** We found a significantly positive correlation (r = 0.57) between the numbers of sequence reads for each diet species and the numbers of individuals for that species identified morphologically. Both data sets contain rotifers as dominant groups, and also contain daphniids, copepods, opossum shrimps, and insects. Calculated functional responses showed that an opossum shrimp *Neomysis japonica* was strongly preferred, while rotifers were not preferred. **Significance:** Our results support usage of the number of reads from metabarcoding as a kind of quantitative measure for species abundance. Calculating the functional responses can allow prediction of future population dynamics of the focal species and its diet species.

DNA metabarcoding of fungal communities in medicinal and edible lotus seeds

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Background: Exhibiting both pharmaceutical properties and edible values, lotus seeds (Semen nelumbinis) have been widely used for decades. However, under proper temperature and humidity conditions, lotus seeds are easily contaminated by toxigenic fungi and their toxic metabolites in the growth, harvest, and storage processes, significantly influencing the quality and safety and posing serious threats to consumers. Therefore, identifying these fungi, especially toxigenic fungi, in lotus seeds using an accurate and sensitive method is necessary and urgent. Recently, DNA metabarcoding, a combination of high-throughput sequencing and DNA taxonomy, has emerged as a fast and easy-to-use approach for microbial communities or species identifications from bulk samples, only requiring a small amount of starting material. **Results:** Four experimental groups, including artificially cultured fungal contamination, mildewed with obvious bacterial plaque, un-mildewed, and surface-sterilized samples, were set up. DNA was extracted, and the ITS locus was amplified using published universal and newly designed fungal primers. PCR products were purified and sequenced on the Illumina MiSeq PE250 platform. For each sample, on average 110 062 ITS sequences were obtained and clustered into operational taxonomic units (≥98% sequence similarity). Results showed that fungal contaminants were present, and abundant fungal communities were detected. The predominant fungal communities were common moulds, such as the genera *Rhizopus*, *Mucor*, *Aspergillus*, and *Penicillium*. Sequences of these four genera occupied 78.2%–97.6% of the fungal communities, and the proportion of *Aspergillus* was much higher in the mildewed samples compared to the normal and surface-sterilized samples. In addition, more than 100 fungal taxa were identified to species level, including toxigenic fungi, such as *Aspergillus flavi*, *Penicillium citroviride*, and *Aspergillus ochraceus*. **Significance:** DNA metabarcoding provides a suitable, practical, and cost-effective approach for rapid monitoring and identification of fungal communities in complex lotus seeds, revealing wide prospects for application.

DNA barcoding of Hong Kong *Ilex* species

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Background: *Ilex asprella* has been shown having good anti-influenza virus activity and *I. pubescens* having excellent anti-inflammatory effect. In Hong Kong and South China, there are 20 *Ilex* species commonly found. We set forth to clarify the relationship of these herbs by DNA barcoding. All of the 20 species were collected, and their DNA was extracted. rbcL, psbA–trnH, matK, and ITS2 sequences were amplified by polymerase chain reaction and sequenced. **Result:** We found that matK was not amplified in most of the *Ilex* species. rbcL was quite conserved and only showed single nucleotide polymorphism in a few nucleotides. ITS2 gave the highest variation, and within this region, a segment was identified for authentication purpose. By using the ITS2 sequences and the Maximum Likelihood model, a phyloge-

netic tree was constructed. The relationship of these 20 species and with other published *Ilex* species was compared. **Significance:** Our study has deepened our understanding of the relationships among the common *Ilex* species found in Hong Kong and South China. After aligning the DNA sequences, specific ITS2 primers that amplify a region of about 200 bp were designed. They may be used to authenticate the medicinal *Ilex* species. By using the specific primers, molecular authentication of the medicinal *Ilex* species can be performed effectively.

DNA barcodes of Nordic Echinodermata

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Background: We prepared 725 specimens of Echinodermata for DNA barcoding with BOLD. The specimens were sampled from 3 to 2560 m depth around Norway, Sweden, the Arctic Ocean, and Bouvet Island. To estimate progress in barcode library species coverage, we revised the list of species recorded from Norway to comply with the taxonomic hierarchy used in the BOLD system. **Results:** In total, 148 species were identified. We obtained 521 sequences, of which 434 were barcode compliant. The barcodes represent 120 species assigned to 128 BINs. Two sequences turned out to be an unidentified Myzostomida associated with *Poliometra prolixa*. The checklist provided by the Norwegian Biodiversity Information Centre has 153 species occurring in Norway. We found specimens of 109 of these species. Seventeen of them failed in barcoding. This gives a species sampling success of 72%, and 61% of the species are barcoded. Among interesting observations are that the northern *Pteraster militaris* (O.F. Müller, 1776) is 98.8% similar to *Pteraster gibber* (Sladen, 1882) from the southern oceans; *Bathycrinus carpenterii* (Danielssen & Koren, 1877) and *Bathycrinus australis* AH Clark, 1907 are similar enough to be assigned to the same BIN, BOLD:AAF3940. Barcode discordance analysis on sequences 300 bp or longer identified 433 concordant records in 96 BINs, 29 BINs with singletons, and 3 discordant BINs with 18 records. In the latter category are the Echinidae *Gracilechinus acutus* (Lamarck, 1816), *Gracilechinus elegans* (Düben & Koren, 1844), and *Echinus esculentus* L. 1758 with some specimens that may be hybrids. *Labidoplax buskii* (McIntosh, 1866) has two clades, suggesting that one of them may be misidentified. Similarly, *Hathrometra tenella* (Retzius, 1783) clusters in two clades, raising some doubt about the synonymy with *Hathrometra sarsii* (Düben & Koren, 1846). **Significance:** Our results are highlighting the importance of barcode data for taxonomic evaluation and biodiversity over wide geographic areas.

Modified primers for DNA barcoding and metabarcoding for invasive freshwater fishes

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Background: DNA barcoding can aid in the rapid and accurate identification of invasive species. However, universal primer sets do not always bind to the targeted 5′-region of cytochrome c oxidase 1 (COI) gene region. **Results:** We tested both specific and degenerate primers for 12 invasive and 16 native fish species from the Volga-Kama River basin, one of the largest river systems in Europe. We found that it is impossible to obtain a usable PCR product for 15%–20% cases due to significant variability in primer binding. We propose shifting the reading frames by 7–10 nucleotides within the locus, to more conservative sites. After testing different combinations of primers, we propose using the following pair: m13_invfishCOI_F+R with slightly modified for Sanger sequencing M13-tails for alien species. Using these primers for freshwater fish increased the PCR effectiveness up to 95% without decreasing the reliability of DNAidentification. A set of primers for metabarcoding (sequence of less than 400 bp)—the pair invfishCOI_mbF+mbR—has been modelled and tested for samples with heavily damaged and fragmented DNA. Usage of this set permitted identification of about 90% of the European alien freshwater fishes and has great potential for semiconductor NGS systems and environmental DNA (eDNA) studies. **Significance:** Using the proposed primers would allow an increase in the effectiveness of alien fish species monitoring in European freshwater waterbodies.

Combining DNA barcodes and genomics to assess if a subtropical river in the Neotropics is acting as a geographic barrier for birds

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Background: The riverine barrier hypothesis proposes that large tropical rivers represent geographic barriers to gene flow for different organisms, leading to population differentiation and, eventually, speciation. This hypothesis was first conceived in relation to the Amazon Basin, an area that includes the largest rivers in the Neotropics. Here, we asses if the subtropical Paraná-Paraguay rivers axis in the Del Plata Basin, the second in importance in South America, acts as a barrier to east-west dispersion and gene flow for birds. We use COI and genomics (ddRADseq) to assess differentiation in seven species that have subspecies defined based on morphological differences between sides of these rivers axis. **Results:** Only one species showed genetic differentiation concordant with the current course of the Paraná-Paraguay axis. Another five species showed population structure with an east-west split that is not concordant with the current location of the rivers axis, but that coincide broadly with the Parana River paleo-channel, suggesting a historical and dynamic role of this river in shaping the observed genetic structure. In one of these species the contact zone determined by nuclear loci was displaced towards the west with respect to the transition suggested by COI, morphology, and behavior. The last species showed weak population structure and lacked a clear association with the rivers axis. The species that showed splits coinciding with the rivers axis differed in the timing of their divergence, suggesting that the diversification process did not occur in a single period of time but instead depended on the biology of each species. **Significance:** This study highlights that the combined use of mitochondrial (DNA barcodes) and genomic data can be useful in testing a classic biogeographic hypothesis. In particular, the significant and dynamic role of the Paraná-Paraguay axis is relevant for understanding the evolution of the birds of subtropical South America.

Mapping the main suture zone between two faunistic supercomplexes of the freshwater fauna in northern Eurasia: Cladocera as a model group

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Background: The water fleas (Crustacea: Cladocera) continue to be an important group for understanding the geographic patterns in freshwater biodiversity. DNA barcoding results can help to identify large-scale phylogeographic patterns at a continental scale, e.g., Eurasia. After intensive collecting efforts in Siberia and Far East of Russia during the last decade, the global phylogeography of the Cladocera of northern Eurasia has become tractable. **Results:** For several cladoceran groups (e.g., *Daphnia magna*, *D. longispina*, *Moina brachiata*, *Chydorus sphaericus*) we found that the mitochondrial phylogroups form two

main faunistic super-complexes (European – western Siberian and Beringian) with the suture zone above the western–eastern Siberia boundary (in the Ob' or the Enisey basins). Such a transition zone is not associated with any apparent geographic dispersal barrier, but its formation may be explained by historical factors such as glaciation. The Beringian region served as a dispersion center for several cladoceran taxa, e.g., towards West and South. Also, several areas of Siberia (e.g., the Altai Mountains and the Transbaikalian region) are sources of relict taxa and haplotypes. Genetic diversity is significantly higher in such regions compared to other regions of Eurasia. **Significance:** Our results generate biogeographic hypotheses for cladocerans of the Palearctic that will be tested by ongoing studies of additional cladoceran taxa.

Advances in DNA barcoding of the Cladocera (Crustacea) of the Far Eastern Palearctic

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Background: The water fleas (Cladocera) continue to be in a state of taxonomic flux. A basic understanding of cladoceran diversity is missing for several geographic regions. The Far East of Russia is among such territories, as there are few studies that specifically study the diversity of eastern Palearctic cladocerans. Here, we use DNA barcoding to assess the anomopod cladoceran diversity in the Far East of Russia. **Results:** We found evidence that the Far Eastern Palearctic is a region of cladoceran endemism. Our analyses revealed putative new taxa within several genera and species groups: *Daphnia magna*, *D. similis*, *D. curvirostris*, *Scapholeberis mucronata*, *S. kingii*, *Moina micrura*, *Pleuroxus* sp., *Alonella exisa*, *Chydorus sphaericus*, and others. These new lineages are candidates for morphological study and provide evidence for revisions. Conflicts between morphology and barcoding for these cladocerans have been successfully resolved. **Significance:** DNA barcoding is an effective initial approach to understand cladoceran biodiversity in a poorly studied geographic region and to identify candidates for revisions using both molecular and morphological methods.

Biodiversity assessment by metabarcoding and quantification of indicator species from oil and gas production infrastructure in North Sea sediments

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The environmental DNA (eDNA) approach and metabarcoding are cost-effective methods suggested for diversity assessment in a variety of environments, including the marine benthos and pelagic zone. Monitoring of the impact related to oil and gas exploration in sediments of the Norwegian continental shelf is today based on the taxonomy of mainly macrofauna. Here, it is suggested to use a set of quantitative assays (qPCR or/and ddPCR assays) targeting lower trophic levels, such as prokaryotic and small eukaryotic taxa. An absolute number of molecular marker gene copy of an indicative taxon needs to be normalized due to local and seasonal variability (inter alia total organic matter content) to be useful. Therefore, we establish a method of normalization of quantitative data. Besides normalization, we focus on the inhibition issue regarding enzymatic reactions (PCR) in environmental samples. According to our preliminary results, digital droplet PCR (ddPCR) does not solve entirely the problem of inhibition. Methods of DNA/RNA extraction, molecular marker selection, a method of elimination of pitfalls and artifacts, and bioinformatic analysis are aspects considered in this study, and they will be presented. This study aims at providing recommendations for best practices and proposing robust molecular assays that the Norwegian operators can use for routine monitoring of the benthos.

Cytochrome c oxidase based DNA barcoding of genus *Barilius* from Jammu region of Jammu and Kashmir State of India

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Background: Most of the small fishes which are treated as unwanted for conventional farming have a good potency as ornamental fishes and are popularly known as aquarium fishes. These fishes are considered as living jewels having different color combinations on their body, attractive body shapes, and numerous fin structures, which make them objects of considerable aesthetic value. Identification of ornamental fishes in Jammu and Kashmir State can be challenging, especially due to different climatic conditions, which include both cold and warm water streams, perennial rivers, lakes, and reservoirs. The present study was the first attempt to apply advanced molecular genetic technique for precise identification of ornamental fishes and to authenticate their diversity in Jammu region. **Results:** Identification of the genus *Barilius* from Jammu region was carried out by using DNA barcoding based on sequencing of the cytochrome c oxidase subunit I gene. A total of 40 fish specimens of both species, i.e., *B. vagra* and *B. bendelisis*, were collected from different parts of Jammu region. The COI DNA barcode sequences were generated by PCR and Sanger DNA sequencing. The sequences obtained were submitted in BLAST for similarity match and were further analyzed using MEGA 7 software. BLAST analysis confirmed the identification of the two species. **Significance:** The species-level identification through DNA barcoding reflects the efficacy of the technique in identifying specimens in the ornamental fish trade. This study will help to mark the potential of the DNA barcoding technique in precise species identification of other faunal groups of the region as well.

The DNA barcode reference library for the Canadian mosses recovered from herbarium specimens

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Background: Mosses (Bryophyta) are sensitive indicators of environmental quality and change. However, their subtle morphology often makes species determinations challenging, even for specialists. By contrast, once a comprehensive, accurate reference DNA barcode reference library is available, DNA barcoding will enable the rapid identification of mosses. Mosses were not a key group in driving selection of the standard DNA barcodes (rbcL, matK) for land plants. Difficulties in the recovery of matK from mosses required the search for an alternative solution for this group of nonvascular plants with a different evolutionary history. **Results:** Two plastid-encoded DNA markers, rbcL and trnL-F, and the second nuclear-ribosomal internal transcribed spacer (ITS2) were selected due to their universality, high variability, and applicability for bryophytes. Moss tissue samples from 2500 herbarium specimens stored at the Canadian Museum of Nature and University of Alberta, representing 900 Canadian species, were processed and sequences analyzed at the Center for Biodiversity Genomics. The standard Sanger protocols for rbcL and trnL-F recovered 94% and 90% of the species, respectively. ITS2 was generated for a subset of 400 specimens using both Sanger and single molecule, real-time (SMRT) sequencing. The two platforms exhibited consistent results with respect to recovery of diagnostic sequence data. The combination of two plastid-encoded DNA barcodes discriminated 60% of all species examined and 100% of the genera. The barcode data

revealed 15 cases of deep intraspecific variation, suggesting the presence of cryptic species. About 5% of the specimens were reclassified based on the barcode results. **Significance:** Using SMRT sequencing will substantially reduce analytical costs for the ITS2 amplicons generated from a large pool of the herbarium specimens. The current reference sequence library is ready for use in the identification of bulk moss samples gathered in ecological surveys, for environmental DNA (eDNA) detection, and as a baseline resource for the molecular identification of Canadian mosses.

Hydrous ferric oxides precipitated from contaminated waters at several abandoned antimony (Sb) deposits — microbiological, mineralogical, and geochemical assessment

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Background: Potentially toxic elements such as arsenic (As) and antimony (Sb) represent dangerous contaminants for the ground and surface water around abandoned Sb deposits in Slovakia. We are therefore doing a complex study on the material from tailings, including mineralogy, water quality, and microbial inhabitants of the contaminated waters. The aim of the work presented here was to observe the chemical composition of iron ochres and the role of the microbial diversity in the mobilization or immobilization of As and Sb from a contaminated landfill. In 2018, As-rich (up to 28.3 wt.%) and Sb-rich (up to 2.7 wt.%) samples of HFO were collected at four localities in Slovakia. All samples were analyzed, after appropriate dilution, for As and Sb. **Results:** In all cases, relatively high concentrations of both contaminants were observed. An Illumina MiSeq sequencing platform was applied to detect microbial species composition of the ochre samples. Here, we present a detailed description of the species composition of the site PE-1 from a sedimentation trap below the tailing impoundment at the abandoned Pezinok deposit. We also compared changes in this microbial composition from samples collected during the summer months of 2011 and 2018. Overall, 945 operational taxonomic units (OTUs) were obtained, of which 880 represented bacteria. The most frequent bacteria were *Gallionella* spp. (19%), *Sulfuricurvum* spp. (10%), *Rhodoferax* spp. (6%), and *Melioribacter roseus* (2%). Compared to 2011, we have witnessed a dramatic increase of iron-oxidizing and sulfur-oxidizing bacteria, and aerobic heterotrophic bacteria were replaced by facultatively anaerobic bacteria. **Significance:** An interesting finding of this study is that even the most heavily contaminated samples are populated by diverse communities of microorganisms. The most common *Gallionella* spp., as Fe-oxidising bacteria, and *Sulfuricurvum* spp., as S-oxidising bacteria, play important roles in Fe-oxides precipitation and therefore have significant influence on arsenic mobility.

The importance of the reference library: discovery of unexpected species in ancient sedimentary DNA

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Background: Metabarcoding has proven to be a powerful tool for the analysis of sedimentary ancient DNA (sedaDNA), due to a higher taxonomic resolution compared to conventional methods and the ability to detect taxa that normally do not preserve well. Metabarcoding, however, amplifies a wide range of target and off-target species due to the usage of generic primers, and most of these off-target sequences in the data are ignored due to either a lack of interest or inability to identify them as a result of missing reference data. **Results:** Here, results are presented for two separate metabarcoding analyses of sedaDNA and some of the unexpected bycatch taxa observed: first, the detection of various worm species in mammalian metabarcoding data from lake sediments in northern Norway (10 700 – 3300 cal. a BP) and the Polar Urals in Russia (24 000 – 1300 cal. a BP); and second, the detection of nondiatom freshwater algae in plant metabarcoding and shotgun data from lake sediments across northern Norway and Svalbard (27 000 cal. a BP – Present). **Significance:** Both the unexpected worm and algae species have a great potential as proxies for environmental conditions such as temperature, moisture, and nutrient availability. These bycatch taxa, and their potential ecological information, could be observed due to available reference barcode data. As more and more reference data are generated for either barcodes or whole organelles, we can expect to identify more species that are currently still hidden in our metabarcoding datasets, which can potentially open up new research questions and possibilities.

A comparison of metabarcoding, metagenomics, and morphotaxonomy of marine benthos — influence of primers, DNA extraction, and reference data

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Background: Routine monitoring of benthic biodiversity is critical for managing and understanding impacts on marine ecosystems from, for example, urban and industrial contamination or extraction activities. However, traditional methods using morphologic identification are prohibitive to increasing the ambition of monitoring programs and typically limited to macroinvertebrates. Metabarcoding instead allows for increased spatial and temporal coverage, and extending surveys to meio- and microbenthos, improving accuracy and knowledge of the consequences of impacts. However, it suffers from difficulties such as taxonomic or ecological classification, poor adaptation to biological heterogeneity, and biased or limited taxonomic coverage. **Results:** We utilised samples from two SSU rRNA metabarcoding studies to assess the influence from environmental DNA (eDNA) extraction and PCR primer choice. The first study targeted sediments near offshore oil platforms in the North Sea and the later estuarine and coastal sediments in the Bay of Biscay. The consequences of different extraction strategies, and of primer choice, were compared, targeting both prokaryotes and eukaryotes. Diversity measures, composition, and coverage were compared to morphotaxonomy data and metagenomes. Results confirmed that several extraction replicates are required to cover most of the eukaryotic diversity in a sample, independent of sequencing effort. Heterogeneity between samples also decreased when extracting eDNA from larger volumes. Community profiles showed strong differences depending on which of the two eukaryotic primer pairs were used, though agreement with morphotaxonomy was similar, and in both cases weak below phylum rank. Metagenomes and manual inspection indicated that this was caused, approximately to the same extent, by primer mismatch, insufficient taxonomic reference data or labelling of the same, and missing eDNA in sub-samples. Prokaryotic community profiles appeared more robust to experimental parameters. **Significance:** While it is neither possible nor desirable to generate metabarcoding results identical to morphotaxonomy, these simple comparisons provide insights into biases influencing metabarcoding, useful for continued standardisation efforts.

Estimation of fungal diversity in temperate and boreal Quebec forests using soil DNA meta-barcoding and identification of ecological variables that influence fungal communities

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Background: Fungal diversity is generally poorly described, and this represents an obstacle for companies working with natural resources and wild edible mushrooms. However, we have yet to understand how fungal community structure and richness are shaped by ecological variables in temperate and boreal Quebec forests. The purpose of this study was to describe fungal diversity and to identify the major drivers of fungal community richness and also to reveal ecological associations of some species of economic interest (43 species). We collected 250 soil samples that covered four bioclimatic domains and sequenced the ITS2 region using Illumina MiSeq. Each stand was also thoroughly characterized (thickness of humus, soil pH, vegetation cover, etc.). We used bioinformatics tools and multivariate analysis to analyse our results. **Results:** For sequence-based identification, we constructed a local ITS-rDNA barcode reference library consisting of 169 barcodes obtained from local samples and 67 from Barcode of Life Data Systems (BOLD). Upon analysis of the meta-barcoding data, we obtained 14 500 operational taxonomic units (OTUs) representing the total fungal biodiversity of each site. The maple grove yellow birch bioclimatic domain had the highest fungal richness. Among the identified OTUs, members of the orders Agaricales, Helotiales, and Russulales were the most frequent. The bioclimatic domain, soil pH, dominant tree species, and surficial deposit type are identified to be the major drivers of fungal community structure. We succeeded to identify in our soil samples 34 species of interest and observed that some species are strongly associated with specific ecological variables. **Significance:** This effort to use meta-barcoding will allow a better characterisation of fungal biodiversity and community structure from soil samples across various bioclimatic domains in the province of Quebec. A clearer understanding of the drivers of soil fungal communities is a key to eventually guide forest management or favor the establishment of specific taxa (e.g., edible mushrooms).

DNA barcode evaluation for the palm family

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Background: The palm family (Arecaceae) is known for its very high ecological and economical value. It is composed of 181 currently accepted genera with around 2600 species. Palm identification is challenging for taxonomist and gardener. As an effective tool for quick identification, DNA barcoding of palms has been little investigated. In this study, we tested three cpDNA regions (matK, trnH–psbA, and rbcL) and nrITS (and ITS2). The efficiency of each marker and their combinations were evaluated based on 314 palm species from 100 genera. **Results:** Due to low PCR and sequencing success rate, ITS was not suggested as a supplementary barcode. The resolutions of rbcL, matK, and trnH–psbA individually are very low. There are too many inversions and insertions/deletions for trnH–psbA. Among the five regions, ITS2 was the most efficient for identifying species. The combination rbcL + matK + ITS2 is the most effective in palm identification (species: 84.6%; genus: 93.3%). By using DNA barcoding, we confirmed 94% seedling samples (86 individuals) to genus level. **Significance:** By producing more than 3000 DNA barcodes, this study greatly enriched the database of palms barcodes. ITS2 is necessary for palm identification with high taxonomic resolution.

Strong mitonuclear discordance in two species groups of sawfly genus *Empria* (Hymenoptera, Tenthredinidae)

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Background: In several sawfly taxa, strong mitonuclear discordance has been observed, with nuclear genes supporting species assignments based on morphology, but the barcode region of the mitochondrial COI gene suggesting different relationships. Because previous studies were based on only a few nuclear genes, the reasons and the degree of mitonuclear discordance remain ambiguous. Here, we studied the taxonomy of two closely related species groups within the sawfly genus *Empria* based on genome-scale ddRAD (double digest restriction-site associated DNA) data together with Sanger sequencing of mtCOI data. **Results:** The ddRAD provided data of 145 512 SNPs in 3 733 285 base pairs for the *E. longicornis* group and 44 573 SNPs in 1 714 773 base pairs for the *E. immersa* group. In the *E. longicornis* group, contrary to mtCOI data, monophyly of most species defined based on morphology is well supported by ddRAD data. In the *E. immersa* group, ddRAD data fail to distinguish current species integrity in many cases, but *E. immersa* and European *E. fletcheri* are supported as separate species in all analyses. STRUCTURE suggests that there are three clearly separated populations: European *E. fletcheri*, *E. immersa*, and the other species together. D-statistic tests reveal some introgression between the species. **Significance:** Based on a genomic data of over a million base pairs and thousands of SNPs, the species limits are congruent (*E. longicornis* group) or partly congruent (*E. immersa* group, but possibly because of unresolved taxonomy) with current taxonomy, in contrast to mtCOI data. Even ignoring current taxonomy, nuclear and mitochondrial phylogenies within both species groups are highly incongruent. The strong mitonuclear discordance can be explained by occasional mitochondrial introgression with limited gene flow of nuclear DNA, a pattern that might be common in haplodiploid taxa with slowly evolving mitochondrial genomes.

When is a bird in the hand worth two in the bush — and when not? Considerations about implementing DNA metabarcoding in legally binding biodiversity monitoring

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Counteracting biodiversity loss is one of the Grand Challenges our century. To meet this challenge, it is essential to have highly resolved data that link biodiversity change to environmental pressures, and to know the effectiveness of countermeasures. DNA metabarcoding is a game changer with respect to data generation. Hundreds of studies, from the deep-sea floor to highest mountains, have shown the unique and numerous potentials of this method. As researchers, we are therefore puzzled by the perceived lack of enthusiasm by policy makers towards actually implementing these exciting new tools, yet there is reason. For legally binding environmental policies, information on the status is typically inferred through the presence and frequency of morphologically identified indicator taxa. Often, sophisticated and decade-long intercalibration routines have been established, especially when considering big European legislation such as the Water Framework Directive, the Habitat Directive, and the Marine Strategy Framework Directive. This work is now paying off at the continental scale, and it is clear what, when, and how to observe and report. Obviously, the set of selected bioindicators is not exhaustive, but the errors associated with them and the way they are assessed are well known. Gambling away this "bird in the hand" for a new, not intercalibrated method under active development makes policy-makers hesitant to change. Furthermore, metabarcoding data do not always meet normative requirements for some of the directives, e.g., predefined taxa lists with abundances. Examples from pioneering projects that are part of the large international COST Action DNAqua-Net

demonstrate possibilities to balance the regulatory needs for constant and intercalibrated methods, and make use of the rapidly developing scientific innovations. The different types of strategic partnerships at national and international levels have worked effectively in transforming our new research findings on biodiversity assessment into practical implementation with the aim of counteracting biodiversity loss.

End biodiversity loss through improved tracking of marine threatened invertebrates

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Background: In today's biodiversity crisis, there is an urgent need to monitor aquatic species in their natural habitats, especially those that may be endangered or elusive. Traditional species observation methods, based on acoustic or observational surveys, are inefficient, costly, and time consuming. Environmental DNA (eDNA) allows us to detect species with a high level of sensitivity. These data can be used to obtain occurrence records and to collect more population information in the field. The aims of this project (2019–2022) are to firstly identify occurrence records and generate a database of distributional data for species of crustacean and mollusks that are data deficient (DD) in Sweden. Secondly, we aim to detect threatened species in Swedish habitats using novel genomic methods (DNA metabarcoding, ddPCR). Finally, based on the new data, we will run species distribution and population models, to improve information on geographic range and population status for threatened invertebrates. **Preliminary results:** Samples will be collected from crustaceans and mollusks determined in collaboration with stakeholders. Occurrence lists will be derived by reviewing existing databases and using molecular occurrence data. Good preliminary data are showing us that red-listed species are detectable in benthic communities using a metabarcoding approach. The DD-classified decapod *Eualus cranchii*, for example, was detected with this method at two locations in Västra Götaland, Sweden in soft mud at 53 m depth and in coarse shell sand at 7–8 m depth. This species is extremely difficult to find with traditional methods. Only 21 occurrences are recorded between 1922 and 2006 in the Analysis Portal. **Significance:** This project promises to deliver numerous occurrence records for endangered species, which will provide new knowledge of marine and freshwater species distributions and population modeling that can be directly used in conservation management. The results will be integrated into current monitoring programs like red-listing and action plans.

Biomonitoring coastal macrozoobenthic colonization of artificial substrates in NW Iberia using DNA metabarcoding

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Background: Marine biodiversity assessment is challenging due to the large-scale biomonitoring programs commonly based on morphological identifications, which are time-consuming and require specialists. High-throughput techniques coupled with standardized sampling strategies accurately improve the ability to monitor marine communities. Artificial substrates deployed in coastal areas may selectively influence and enhance the colonization of macrobenthic species, providing an alternative to easily and routinely assess biodiversity. Here, we used DNA metabarcoding to monitor coastal macrozoobenthic communities and investigate the short-term and seasonal patterns of colonization in artificial substrates. In order to do that, three different types of artificial substrates were used: slate, polyvinyl chloride, and granite. In December 2016, 16 replicates of each substrate were randomly deployed close to the dock of Toralla Island (Spain), and after 3, 7, 10, and 15 months, four replicates of each substrate were randomly removed (3 replicates for morphology and 1 for DNA metabarcoding). The mobile and sessile fauna were separated and preserved for subsequent analysis. DNA amplification was performed for an internal region of the COI barcode (~313 bp). **Results:** Compared to morphology, DNA metabarcoding retrieved more taxa and higher taxonomic resolution in all substrate/time combinations. However, some of the species identified with morphology were not detected using COI (e.g., *Ciona intestinalis*). Furthermore, a different recruitment time to colonize the substrates was evident for some taxa (e.g., *Hiatella arctica*) as well as variations in taxonomic diversity among substrates and seasons (e.g., bryozoans in granite and slate). **Significance:** In general, DNA metabarcoding detected a higher number of taxa, and with more resolution, compared to traditional morphological tools. However, the two methods applied were somewhat complementary in their ability to detect benthic species, and both should be used to avoid missing relevant taxa. The results also illustrate the influence of substrate and season in the recruitment of zoobenthos.

ITS2 barcode would be an effective method for the identification of proprietary Chinese medicine sanqi tablet

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Background: Sanqi tablet is widely used in clinical practice for the prevention and treatment of cardiovascular and cerebrovascular diseases in China. Sanqi tablet is a proprietary Chinese medicine (PCM) made from dried roots and rhizomes of *Panax notoginseng* (Burkill) F.H. Chen ex C.H., which are ground into fine powder form, then mixed with excipients to make granules and finally compressed into finished products. However, the market of sanqi tablets has some quality problems due to the similar characteristics of *Panax notoginseng* and its adulterants when using traditional identification methods. The objective of this research was to develop a DNA-barcoding method for PCM identification and to evaluate the applicability and reproducibility of this approach across different laboratories. **Results:** The success rate of DNA extraction and PCR amplification was 100%, and the electrophoresis bands were clear and bright. Single nucleotide polymorphisms (SNPs) were detected, and the results showed that the method successfully identified all the samples, which mixed the adulterants in a certain proportion to participate in *Panax notoginseng* powder. We repeated the experiments six times to investigate the repeatability, and the intermediate precision was investigated by three different people, and the reproducibility was investigated in three different laboratories. We also investigated the stability of the method by examining the sanqi tablets produced in different places and different batches of sanqi tablets produced in the same place. The method was found to be highly reproducible and sensitive enough to identify species present in a mixture at 10% dry weight content. **Significance:** This study confirmed that the ITS2 barcode region would be a reliable and stable method to quickly and effectively identify sanqi tablets and their counterfeit products. Meanwhile, this research provides enlightenment for the identification problem of complex products like PCM, especially for market circulation management.

DNA barcoding of West African birds

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Background: DNA barcoding works well in birds; large surveys of the Nearctic, Palearctic, and Neotropical avifauna have documented that

more than 95% of taxonomically defined species can be identified with a DNA barcode. The few cases of discordance between DNA barcodes and current taxonomy can be attributed to poor taxonomy, the lack of a barcode gap, or species with an internal split in the barcode region. Here, we present the first DNA barcode survey of the Afrotropical avifauna with the aim of comparing the barcode gene tree with current taxonomy. More than 200 species in the West African countries of Nigeria and Cameroon were included. **Results:** Our results confirm previous results from other geographic regions that unique BINs match current taxonomy in about 95% of all cases. More than 15 BINs had internal barcode splits, which in most cases could be explained by vicariance. These BINs require further taxonomic scrutiny for correct species delimitation. Certain groups, like *Illadopsis* and *Cisticola*, are difficult to distinguish morphologically but show well-defined barcode clusters. **Significance:** We conclude that DNA barcoding is a useful tool for species identification of West African birds. Our study highlights some enigmatic taxonomic clusters for further study of species delimitation.

DNA metabarcoding for community profiling of mosquitoes in Sweden

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Background: Several mosquito species are vectors for pathogens infecting humans and animals, making it important for the prediction of spread of disease to know mosquito species distributions. Mosquito species identification has traditionally relied on morphological identification, but DNA barcoding of the mitochondrial COI gene has gained popularity. By using next-generation sequencing (NGS), this method can be further developed into metabarcoding to identify several species in a sample simultaneously. **Results:** We created a database of Swedish mosquito COI sequences by individual Sanger sequencing of 29 mosquito species from Sweden combined with available sequences from GenBank to cover species recently documented in Sweden and possible invasive mosquitoes. Comparison showed that this marker reliably distinguishes 41 of 56 sequences to species and the remainder to species group. We have further developed a method to identify mosquitoes in communities by amplifying the COI gene with four independent primer pairs, sequencing the amplicons by NGS, and using bioinformatic methods to quantify relative abundance of each mosquito species in the sample. Using four primer pairs and combining the results minimizes primer bias and increases number of species detected. By using communities assembled from morphologically identified mosquitoes, we tested the accuracy of the metabarcoding method, and it can detect species represented by a single mosquito in a population of 100, has a detection rate of 80% of species, and the estimated population structure reflects the input sample, making the method useful also for semi-quantification of species. The metabarcoding technique was used to survey the seasonal dynamics of mosquito populations in four locations during 1–3 seasons, showing similar trends in species succession during the season. **Significance:** DNA barcoding using the COI marker provides species-level information for most northern European mosquito species. Metabarcoding can be used as a high-throughput identification technique, allowing studies of populations throughout the mosquito season.

DNA barcoding projects of the native vascular plants in Korea

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Background: Building a DNA barcode database at the local scale is crucial for conservation biology and the practical use of biological resources in many fields. Recently, misuses, mixed uses, forges, and falsifications of biological material have grown rapidly due to challenges in identification. In 2008, the National Institute of Biological Resources (NIBR) established a DNA barcode database of Korean vascular plants for the following purposes: (*i*) to discover new or unrecorded cryptic species; (*ii*) to discriminate morphologically indistinguishable taxa to secure food safety, and the authenticity of plant-derived biomaterials; and (*iii*) to provide solid taxonomic information for ecological, evolutionary, and environmental studies. **Results:** We examined rbcL sequences of all plant genera in Korea (1122 genera) and constructed a genus-level reference library for Korean vascular plants. Neighbor-joining analysis of 1122 Korean vascular plants in the rbcL library revealed a high resolution of genus-level taxonomic relationships (>95%). Additionally, we built a barcode reference library containing standard barcode regions, including rbcL, matK, psbA–trnH, and ITS, for 2795 species (62% of 4518 species listed in the Database of vascular plants of Korea). The conventional DNA barcodes ambiguously and(or) falsely discriminated species in plant materials with low resolution, e.g., genus *Artemisia*. For those problematic taxa, we applied the advanced technique Hyb-Seq and developed species identification markers from multiple orthologous genes. A maximum-likelihood inference with the newly developed markers exhibited significantly high resolution (100%) at the species level in Korean *Artemisia*. **Significance:** Our results indicated that the rbcL reference library is very effective in identifying Korean vascular plants to the genus level. For species-level identification, we found that identification markers collected across multiple orthologous genes through the Hyb-Seq technique improved the resolution significantly. The barcode markers we developed may well be practically applied to discriminate traditional medicinal herbs in Korea, such as *Artemisia* species.

Comparison of capture array, metabarcoding, and shotgun sequencing in recovering mammalian eDNA from contemporary soil samples

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Background: Ongoing rampant environmental change increases the urgency in developing better survey tools to make accurate predictions for such conservation flagship species' distribution and movement over time. Environmental DNA (eDNA) is noninvasive and not constrained by the visual encounter of species. It offers possibilities to explore mammal occurrences in previously inaccessible areas. eDNA metabarcoding has been widely applied in detecting aquatic macroorganisms. However, its applications in terrestrial ecosystems, especially targeting mammals, have been relatively less represented. Capture array and shotgun sequencing have succeeded in recovering mammal DNA from ancient bones and lake sediments. While promising, there has not yet been a direct comparison between these three methods for detecting terrestrial mammals. **Results:** We directly compared DNA metabarcoding, capture array, and shotgun sequencing on terrestrial eDNA samples collected in the Merced Vernal Pool Reserve, UC Merced, USA, where a complete mammal survey is available. Metabarcoding primers 12SV5 and MamP007 were chosen to maximize mammal specificity. The capture array covered 75 mammalian whole mitochondrion sequences spanning the phylogenetic tree. We found that Actinobacteria and Proteobacteria constitute 92% of sequences obtained, while only 0.2% of sequences were assigned to Mammalia in shotgun sequencing. Both capture array and DNA metabarcoding could detect *Bos taurus*, an abundant and large mammal at the site, while shotgun sequencing could not. It was the only mammal we recovered at the site. **Significance:** Our result shows that capture array and DNA metabarcoding are able to detect terrestrial mammals, but not full survey observations. It is possibly caused by the scarcity of mammal DNA in surface soil. With ongoing analysis, we are trying enrichment techniques to concentrate mammalian sequences by applying restriction enzymes specifically targeting microbial groups be-

fore barcoding efforts. This technique holds potential for rapid biodiversity surveys in remote area and ancient sediments where traditional observations are not available.

Mapping California biodiversity using remote sensing and community science eDNA surveys

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Background: As one of the world's biodiversity hotspots, California's unique ecosystems are under threat from ongoing global change. Baseline biodiversity data provides valuable information for effective resource management in the face of global change. The California Environmental DNA (CALeDNA) Program, launched by the University of California Conservation Genomics Consortium, is a community science initiative aiming to monitor all facets of California's biodiversity and provide critical baseline biodiversity data using environmental DNA (eDNA). We invite community scientists to collect soil and sediment samples across California's diverse ecosystems and in return share the metabarcoding results online with the public and resource managers. **Results:** We have accumulated over 3000 georeferenced samples across California since project initiation in 2017. In total, 278 samples were analyzed through multi-locus DNA metabarcoding targeting all kingdoms of life. Sequence filtering and taxonomy assignments were performed using Anacapa Toolkit. A total of 16 118 taxonomic entries were recovered. We also compiled a list of remote sensing derived environmental variables, ranging from bioclimatic variables to vegetation cover, human impact, soil properties, and habitat classifications layers. Community ecology analysis revealed distinct diversity patterns across different habitats. We further explained the effects of environmental variables on such community assemblages using gradient forest modelling. We found that a reduced set of 10 environmental variables was able to predict 33% of total presence/absence patterns at the family level. Vegetation coverage and elevation are the top predictors for species distribution in California. **Significance:** We demonstrate the power of harnessing benefits from the rapidly evolving Earth observation technologies and high-throughput sequencing advances in community science programs for analyzing biodiversity patterns.

Evaluation of species-specific primers and extraction methods for eDNA-based detection of the American bullfrog

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Background: Environmental DNA (eDNA)-based methods are becoming routine in biomonitoring projects. Active detection using eDNA and quantitative PCR (qPCR) with species-specific primers can detect aquatic invasive species at low abundance. Primer specificity is crucial in such assays because amplification of off-target species can result in false-positive signals, while failed amplification of target species can lead to false negatives. Limited studies have compared primer specificity across different geographical regions. Additionally, it is also important to evaluate extraction methods before application in biomonitoring programs to ensure abundant recovery of target DNA. **Results:** To develop an eDNA-based qPCR assay for American bullfrog (*Rana catesbeiana*) monitoring in Beijing, China, we designed a primer and probe set targeting bullfrog DNA, and compared its species specificity using in silico PCR and in vitro qPCR. In silico PCR results suggested that only a small number of amphibian species in North America were potentially amplifiable. In in vitro qPCR, only bullfrog DNA can be amplified, while DNA of other native anurans in Beijing cannot. However, a previously reported primer pair developed for a biomonitoring project in France specifically targeting bullfrog amplified a common native frog in Beijing in vitro. Three commonly used eDNA extraction methods—the phenol-chloroform-isoamyl alcohol (PCI) method, cetyltrimethylammonium bromide (CTAB)-based extraction, and Qiagen's DNeasy Blood and Tissue Kit—were examined as well. Using water samples from bullfrog tadpole-rearing laboratory aquariums, we found that the CTAB method significantly outperformed PCI or the DNeasy kit in bullfrog DNA yield and was more time and cost-effective. **Significance:** We report a useful primer-probe set and extraction method evaluation for eDNA-based bullfrog biomonitoring. We also demonstrate that species-specificity of existing primers for targeted detection should be carefully evaluated before their application in a new ecosystem.

Starting a DNA barcode reference library for Chinese nonbiting midges (Diptera: Chironomidae)

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Background: Chironomids, also called nonbiting midges, including over 6,300 described species worldwide, are important bioindicators for monitoring freshwater ecosystems. A comprehensive DNA barcode reference library of Chinese chironomids is needed to address the following challenges: (*i*) species-level identification in chironomids using morphology sometimes is difficult, particularly in the immature life stages; (*ii*) the linking of the larval stages with the adult forms by rearing is time-consuming and not always successful; and (*iii*) a database for metabarcoding for the monitoring of freshwater ecosystems in China is incomplete. Hence, we are developing and improving the DNA barcode reference library of Chinese chironomids based on museum collections and newly collected samples, and have generated and uploaded more than 1200 COI barcodes of chironomids to BOLD from our own laboratory, along with their individual images, geo-references, and other relevant laboratory data. **Results:** Currently, about 1500 barcodes from China representing ~400 chironomid species of 82 genera within five subfamilies have been generated and submitted to BOLD and GenBank. In general, most morphospecies can be differentiated by DNA barcodes with an average threshold at 4%–5%, higher than for the BIN algorithm. Additionally, the immature stages are associated with adults by DNA barcodes accurately. Moreover, a number of potential cryptic species and misidentifications are uncovered by DNA barcodes. **Significance:** Large-scale sampling is required for a comprehensive DNA barcode reference library of chironomids in China, which will be widely used for scientific research, education, and aquatic conservation.

Environmental DNA metabarcoding enables early detection of non-indigenous aquatic invertebrates and fish in Belarussian rivers

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Background: Many rivers in Belarus find their origin across the national border in the Baltic and Black Sea basins. Within Belarus, these rivers are secondarily connected via canals. The interconnectivity of rivers originating from different areas within Europe form the Belarussian part of the Central European invasion corridor. Documenting the introduction and spread of non-indigenous species within Belarus is of national and European importance. Furthermore, global climate change is enhancing the spread of non-indigenous species into Belarus from Kiev (Ukraine) and Kaunas (Lithuania). While monitoring

of non-indigenous aquatic invertebrates and fish started in 2006–2007 by standard hydrobiological and ichthyological surveys, novel methodology enhancing early detection probability is required for yet-to-be-established invaders. Here, we compared standard monitoring and environmental DNA (eDNA) metabarcoding techniques for the detection of new non-indigenous invertebrates and fish in Belarus. **Results:** eDNA sampling was performed at 12 sites on nine rivers across Belarus. These sites represent a subset of areas regularly monitored by standard hydrobiological and ichthyologic surveys. Within each site, nine samples were taken, covering three habitats. The volume of water samples filtered through SterivexTM filters ranged from 250 to 750 mL, depending on turbidity of the water column. Two established metabarcoding assays targeting the 16S rDNA fragment were used to detect fish and crustacean diversity. A near-complete barcoded local reference database allowed for species detection in most instances and enabled a species detection comparison between standard hydrobiological and ichthyological surveys and eDNA metabarcoding. **Significance:** This is the first effort to provide eDNA metabarcoding-based monitoring of alien invertebrates and fish in the rivers of Belarus, with the big hope to implement this approach into the national monitoring program. Moreover, the results of this study will be used to update the national checklist of non-indigenous aquatic invertebrates and fish of Belarus.

An international genomic reference database for medicinal plants

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Background: DNA barcoding technology has been widely used in research and real-word applications and has played a pivotal role in ensuring quality and safety of botanical materials. With the maturity of the single-marker based technology and the wide adoption of the next- and third-generation DNA sequencing technologies, it is time to consider extending the single-marker based identification to a genomic markers based approach. Since March 2017, the Institute of Medicinal Plant Development and Illumina Company have signed a collaboration agreement to start a "1000 Medicinal Plant Genomes" project. The initial strategy is to use a genome skimming strategy to obtain the plastome sequences of 1000 Medicinal Plant Genomes in three years. Here, we would like to describe the progress of this project. **Results:** We have collected and annotated ~500 medicinal plants species. The genomic DNA of ~300 medicinal plant species has been sequenced. The corresponding plastome sequences have been assembled and annotated. Several applications, such as bioinformatic tools for simultaneous determination of multiple components from biological mixtures and a block-chain based tracking system, have been developed. On the other hand, we have also run into several problems, such as ensuring the quality of the voucher samples and fair intellectual property sharing. **Significance:** As the success of this project will require the participation of researchers, industrialists, and governmental officials alike around the globe, we would like to call for a close collaboration among all practitioners involved in DNA-based identification of medicinal plants.

Comparing the effectiveness of beetle biodiversity monitoring through DNA metabarcoding and morphological approaches

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Background: Human-induced disturbance has spread into most ecosystems, including forests. Beetles are sensitive ecological indicators of the impacts of forest disturbance, including forestry. Beetle biodiversity studies have traditionally been conducted through morphology-based identification and counts of species in samples, which is extremely labour intensive and costly. DNA metabarcoding is now possible for beetle biodiversity studies, yet has rarely been applied in the context of sustainable forest management. One of the major reasons is the coarse correlation between species biomass and sequencing reads, hindering estimation of species' abundances. In order to inform forest management, accurate identification of beetle species and relative abundances is fundamental. **Results:** In this study, I will present results of a comparison of beetle biodiversity through DNA metabarcoding and morphological approaches. Rather than using mock samples, I used 24 real world bulk beetle samples collected from unlogged mature forest and nearby regeneration forest habitats. First, I tested the performance of DNA metabarcoding in species identification and abundance estimation with diverse beetle samples. Second, I performed ecological statistical analysis to assess whether estimation of species abundance from DNA metabarcoding is sufficiently sensitive for accurate assessment of the impact of forest management. Beetle DNA metabarcoding can be calibrated with morphological approaches and thus can be applied in the context of ecological analysis. **Significance:** Beetle DNA metabarcoding is promising for large-scale biodiversity monitoring. It is also expected to have similar accuracy for species abundance estimation and thus on informing forest management. Our study will investigate the usefulness of DNA metabarcoding for accurate beetle biodiversity monitoring with calibration through morphological assessment. Beetle DNA metabarcoding can thus facilitate forest biodiversity monitoring and sustainable forest management.

Zhen-Tracking: a block-chain based tracking system based on genetic fingerprints

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Background: The Chinese Food and Drug Administration has issued the guidance for constructing tracking systems to ensure the quality and safety of drugs. However, the classical tracking system has difficulty for wide application due to the lack of trust among the participants. Recently, the block chain technology has been used in many areas due to its decentralized, distributed, and immutable characteristics. **Results:** Here, we constructed a tracking system based on genetic fingerprints of medicinal plants and the block-chain technology, called Zhen-Tracking, using the Python programming language. The system includes a mobile client and a block-chain based data storage system. The client app can be used by everyone, for example, those involved in the production, sale, and consumption of the products by scanning the QR code containing the genetic information of the products to be tracked. For the producers, they provide the genetic fingerprints of the plant materials to construct a QR code, whose information will be stored on the chain. For the sale persons, they scan the code to store the relevant circulation information on the block chain. For the consumers, they can scan the QR code to obtain the information about the sources and circulation paths of the products. The Zhen-Tracking system has several advantages, such as large capacity, high degree of security, free of centralized management, etc. **Significance:** In a decentralized global environment, the system will be invaluable for ensuring safety and efficacy of products of health, food, and traditional medicines.

Using complete plastomes and nuclear ribosomal DNA sequences as the next-generation DNA barcodes in *Panax* (Araliaceae)

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Background: The complete plastomes and the nuclear ribosomal DNA sequences were proposed as the candidates of the next-generation DNA barcodes for plant species discrimination. However, their validity

still lacks comprehensive evaluation. **Results:** We carried out a case study in the economically important but phylogenetically and taxonomically difficult genus *Panax*, including a large data set of the plastomes and nuclear ribosomal DNA sequences from multiple accessions per species. Our data largely improved resolution in phylogeny and species identification in *Panax*, in contrast to any previous studies using single- or multiple-locus DNA sequences. The progress may provide new insights into speciation, lineage diversification, and range formation in the genus. However, both data sets failed to completely resolve the phylogenetic relationships in the *P. bipinnatifidus* species complex, and only half of species within it were recovered as monophyletic groups. **Significance:** The results suggest that both complete plastomes and ribosomal DNA sequences are not powerful enough to reconstruct a robust phylogeny and reach a full species identification, especially at low taxonomical levels or among closely related species. To achieve the ultimate big gains in resolving discrimination power in plant barcoding, the complement of substantial numbers of nuclear markers is indispensable.

Multitudinous species identification for bulk samples based on high-throughput sequencing using ITS2 and full-length COI, rbcL, and matK with a PCR-free approach

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Background: Species assessment for mixed animal and plant ingredients is a hot topic not only for ecologists but also for herbal and food product supervisors. However, the application of the DNA metabarcoding approach was previously limited mainly by its dependency on PCR efficiency. Moreover, these products are always amplified using mini barcode primers because of their highly degraded DNA properties and will also reduce the taxonomic resolution compared with that of long, standardized barcodes. **Results:** Here, we developed new high-throughput sequencing pipelines without PCR amplifications that can capture ITS2 and full-length COI, rbcL, and matK sequences for biological ingredients. First, total DNA of bulk samples was enriched and then put to Illumina sequencing with 250–270 bp libraries. Different sequencing amounts (3 G, 6 G, 9 G, 12 G, 24 G) were investigated to determine the appropriate sequencing amount. Second, short reads of ITS2, COI, rbcL, and matK were selected using the BLAST procedure with simplified reference databases, where sequences lower than 90% similarity within each genus were removed and only contain the longest representation sequences. Target reads for each locus were assembled separately using an overlap-layout-consensus based software rather than the popular de-bruijn-graph software. The above method was trained using self-made reference herbal products and verified with 10 batches of real samples purchased from marketplaces. Full-length COI sequences for all three animal ingredients were successfully obtained, whereas ITS2 and full-length rbcL and matK sequences for all 10 plant ingredients were successfully assembled except for two highly processed specimens. At the same time, some contaminants and substitutions were detected that may pose serious health risks to consumers. **Significance:** The ability of the new PCR-free pipelines to obtain ITS2 and full-length COI, rbcL, and matK sequences can overcome the limitation of PCR efficiency and provide more information because full-length sequences were obtained.

Molecular detection of invasive alien species using real-time quantitative PCR, a tool to advance management strategies

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Background: Invasive alien species (IAS), particularly pathogens and pests, are a significant threat to biodiversity. Their introduction and establishment can cause major damage to natural ecosystems and significant economic losses. Many species of concern are expanding or shifting their distribution as a result of climate change, anthropogenic impact, transportation advances, and increased global trade. The early detection of alien species, at their invasion fronts or points of entry, is critical in limiting their establishment and subsequent spread. Here, we validated and used real-time quantitative Polymerase Chain Reaction (qPCR) assays to identify IAS directly from vectors or environmental samples. We also explored the use of novel technology that allows molecular detections at the point-of-need. **Results:** Using TaqMan™ qPCR species-specific assays, we successfully detected invasive species of Canadian regulatory concern in the early stages of their invasion. Our results support the advantage of molecular tools to detect IAS at low densities and to reveal recently invaded areas. We also demonstrated how molecular detections can directly instruct regulatory agencies on management policies. **Significance:** The use of molecular tools and well-populated molecular databases can provide rapid, cost-effective, and accurate identifications of IAS at any life stage, and from a variety of sample sources (e.g., forensic remains, bulk samples, and environmental DNA). The prompt and reliable detection of IAS is crucial to implement management decisions such as quarantine or control strategies. Lastly, we highlight the potential of using onsite screening tools to improve and accelerate the implementation of management strategies.

Investigating the value of gardens for providing floral resources to pollinating insects

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Background: Pollination is a key ecosystem service that has significant economic value as well as facilitating wider ecosystem function. There has been a considerable decline of pollinators in recent years, owing to pressures such as habitat fragmentation, climate change, pests, and disease. As floral resources are a limiting factor of pollinator abundance, gardens could play a key role in alleviating pollinator declines by providing a wealth of native and non-native resources and increasing floristic diversity throughout the year. The aim of this study is to identify foraging preferences in order to provide appropriate resources. **Results:** We have sampled 72 bees (honeybees, solitary bees, and bumble bees) and 85 hoverflies across eight areas of varying habitat type within the National Botanic Garden of Wales and Waun Las National Nature Reserve, Wales, UK. Honey has also been sampled monthly between April and September from an apiary at each site. Foraging will be investigated using DNA metabarcoding of rbcL and ITS2 markers. The area surrounding the study sites has been surveyed during the same period that pollinators were sampled, to create a record of what floral resources are available at each time period, and how much of the floral availability is utilised by the pollinators. We will investigate inter- and intraspecific partitioning of floral resources and whether native or non-native plants are preferred. **Significance:** Extensive lists name pollinator-friendly plants that can be planted to aid biodiversity; however, these lists are inconsistent, and only a limited number is based on clear scientific evidence. There are doubts about their attractiveness to pollinators, and whether they are pesticide free. The results of this research will be delivered to gardeners and policy makers to aid in pollinator conservation management and used to recommend plants for the creation of seed mixes which target specific species or groups.

Forest community assembly is driven by different strata-dependent processes along an elevational gradient

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Background: Integrating taxonomic, phylogenetic, and functional diversity can provide insights into the relative contribution of ecological and evolutionary processes in community assembly. Forest ecosystems are structurally complex and consist of different strata (overstory and understory); however, few studies assess how the environment influences scale- and strata-dependent community assembly processes. Here, we examined the relative effects of environmental filtering, biotic interactions, and stochastic processes driving community structure across scales and strata along an elevational gradient in a hyperdiverse forest on Yulong Mt., Hengduan Mountains, Southwest China. We sampled 320 co-occurring plant taxa from forest plots along a 1200 m elevational gradient. Using a framework that incorporated phylogeny based on DNA barcodes (rbcL, matK, and ITS), traits, and environmental variables, we determined the importance of biotic, abiotic, and stochastic processes in structuring communities across forest strata at neighbourhood (tree: 10 m × 10 m; shrub: 2 m × 2 m; herb: 1 m × 1 m) and community (20 m × 50 m) scales. **Results:** We demonstrated that different forest strata were shaped by distinct mechanisms along the elevational gradient. At the neighbourhood scale, we found that environmental filtering by temperature and soil water content reduced tree functional-phylogenetic diversity at both ends of the elevational gradient, while communities with higher species diversity exhibited an overdispersed functional-phylogenetic structure at mid-elevations. At both the neighbourhood and community scales, our results were consistent with facilitative interactions among distantly related species, promoting the overdispersed phylogenetic structure of shrubs at high elevations despite low species richness. However, stochastic processes dominated assembly patterns of herbaceous communities, although there was some influence from local light conditions. **Significance:** Our findings underscore multiple assembly processes in forests and for different strata. Further, incongruent patterns for overstory and understory communities shed light on how multiple factors simultaneously structure forest communities.

Appropriate genetic marker for genus *Cryptosporidium*

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Background: Cryptosporidiosis, a protozoan disease caused by representatives of the genus *Cryptosporidium*, occurs worldwide and takes a major toll on the health of infants and toddlers in developing countries (poor sanitation), and may also cause debilitating disease in immunocompromised individuals. The application of molecular methods for the determination of species and genotypes of *Cryptosporidium* is of great importance because the genetic characteristics of different species are crucial for the determination of the accurate diagnosis and consequently for its treatment, prevention, and environmental monitoring. Most common molecular markers for *Cryptosporidium* spp. are genes encoding small ribosomal RNA (18SSU rRNA), 60-kDa glycoprotein, COWP, and heat shock protein. Our aim is to find a DNA barcode marker for the precise identification of species and genotypes of the genus *Cryptosporidium*. **Results:** As a universal marker for the identification of *Cryptosporidium* species, we chose a partial sequence of the SSU rRNA gene. The problem with this approach is the presence of mixed infection (we used Sanger sequencing). We analysed faecal samples of pigs, cattle, and dogs from eastern Slovakia. After DNA extraction using the DNA-Sorb B nucleic acid extraction kit (Amplisense, Russia) with a pre-extraction step for the disruption of the oocyst's wall using a Bead beater homogenizer, we continued with DNA detection by nested PCR with specific primers for SSUrRNA (VKSSF1/VKSSR and VKSSF2/VKSSR2). Using SSU rRNA, we amplified four different species of *Cryptosporidium*, namely *Cryptosporidium parvum*, *C. scrofarum*, *C. bovis*, and *C. muris*/*C. andersoni*. **Significance:** To find a universal marker for the genus *Cryptosporidium* is still a problem. In our study, we had a problem using SSUrRNA to discriminate between *C. muris* and *C. andersoni*. For this species, it is still necessary to use specific primers for another gene (gene encoding 60-kDa glycoprotein). The same problem occurs for species *C. parvum* and C. *hominis,* where 18S rRNA does not give us the precise discrimination between these two species.

Metabarcoding of bulk arthropod samples reveals vertebrate diversity

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Background: World-wide, thousands of bulk arthropod samples are collected for monitoring programs, conservation efforts, and ecosystem assessments. The taxonomic contents of such samples can be efficiently assessed using metabarcoding coupled with high-throughput DNA sequencing. On the other hand, targeted collection of invertebrates, specifically those known to eat flesh, blood, faeces, and decaying organic matter, followed by DNA analyses of the vertebrate taxa in their gut contents, can provide information of vertebrate diversity (invertebrate-derived DNA, iDNA). Such targeted collection of vertebrate-eating invertebrates followed by DNA analysis has offered a new and promising tool to complement traditional vertebrate monitoring methods, something of great value in ongoing biodiversity monitoring efforts. This leaves the opportunity to merge these two study fields, that is, to use iDNA methods to detect vertebrate DNA in bulk arthropod samples, without targeting a specific invertebrate and thereby optimising the biodiversity information gained from bulk arthropod samples. **Results:** Metabarcoding of bulk arthropod samples collected in Brazil (103) and Tanzania (162) revealed a total of 30 vertebrate taxa representing three mammalian orders, one amphibian order, and three bird orders. Detected taxa were within or close to their known geographical distributions. The vertebrate taxa were different, and therefore there were no overlapping taxa detections with the two metabarcoding primers used. **Significance:** This study demonstrates that with a relatively small additional investment, vertebrate diversity information can be obtained from bulk arthropod samples. Therefore, once having collected and extracted bulk arthropod samples, researchers can use bulk arthropod samples to detect vertebrate diversity. This could be of relevance to ecological assessment and monitoring programs, by serving as a supplement to traditional survey methods and can be of particular interest in larger projects where bulk arthropod samples have already been collected and DNA extracted.

Mapping phylogeographic endemism of amphibians in a Brazilian hotspot: implications for conservation management decisions

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Background: Amphibians are an important component of the world's vertebrate fauna, with nearly 8000 species and with represen-

tatives found in virtually all terrestrial and freshwater habitats, in all but the coldest and driest regions or the most remote oceanic islands. They are also considered excellent models to study adaptation and indicators of environmental change. The number of known species of amphibians has grown by nearly 95% in the last three decades. Unfortunately, the rapid increase in knowledge of amphibian species diversity is coincident with a massive and global decline in amphibian populations. As such, it is urgent that the scientific community redoubles its efforts to gather information on amphibian diversity in order to understand the full extent of the diversity and develop strategies to stem the decline and extinction of these species. **Results:** In this study we used DNA barcode and distribution modelling information to enhance the description of biological diversity of amphibians in the Brazilian Atlantic Forest (AF). We DNA barcoded nearly 4000 individuals from 380 species and mapped the genetic diversity across the AF distribution range. We found that ~25% of nominal species presented very deep divergent lineages, suggesting that amphibian diversity in the Neotropics is still highly underestimated. We found endemic lineages throughout the distribution of the biome, and also regions with high endemism represented by mountains regions or the transition zone in between bioclimatic domains. **Significance:** Our data indicate that future conservation management strategies need to incorporate genetic diversity within individual amphibians species, protecting populations in different parts of a species' range and taking into account the richness of species not yet described. It is also necessary to discuss the formation of new protected areas to preserve lineages and endemic species that are seriously threatened by land use.

Regulatory applications of barcoding in the Canadian Food Inspection Agency's Plant Health Program

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The Canadian Food Inspection Agency (CFIA) is Canada's largest science-based regulator, with responsibility to deliver all federally mandated programs for food safety and plant and animal health. In order to meet these obligations, CFIA relies on sound science as the basis of its program design and regulatory decision-making. Rapid and accurate identification of regulated plant pests is critical for design and delivery of preventative and risk management strategies. Genomic tools such as DNA barcoding can enhance the identification of target species in a regulatory context by identifying species regardless of life stage (i.e., juvenile vs. adult) or sample composition (i.e., "bucket of bugs"), and by distinguishing target species from closely related and(or) visually similar species. These tools can be applied to commercial imports of grain or seed but also in support of export activities when relevant and available. CFIA research scientists have ongoing collaborations targeted at using barcoding to support the development of diagnostic tools and methodologies for early detection, surveillance, management, and science-based decision-making. Current activities and collaborations to apply barcoding to the regulatory activities will be discussed and specific applications presented.

Revealing hidden diversity of planktonic organisms in an Indonesian reef system using environmental DNA

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Background: Planktonic organisms are important drivers of productivity in marine ecosystems. Data suggest that there is likely an enormous amount of hidden diversity in planktonic communities on coral reefs, regardless of their location, not to mention the microcommunities within corals themselves. However, there is a current lack of data in Indonesia, and many of these important species, which underpin coral reef fisheries food webs and thus coastal food security, remain to be discovered. In this study, we strategically sample planktonic, sessile, and motile organisms along gradients of fishing pressure and fisheries management in coral reefs of Lombok Island (Nusa Tenggara Barat) and Raja Ampat Islands (Papua). **Results:** We used a suite of methods, including environmental DNA (eDNA). Water filtering of 4 L occurred using 20 and 0.4 μm filters at surface, mid-water, bottom, and sediment for each site. We confirm that the most biodiversity originates from benthic planktonic organisms. **Significance:** These results suggest that sedimentary eDNA could be used to estimate the ecological structure of the entire pelagic community in the coral reef ecosystems.

Detecting habitat and population structure of the endemic Halmaheran walking shark (*Hemiscyllium halmahera*) using a molecular approach

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Background: An important key in understanding marine ecosystems, and the implementation of science-based rules, is the development of environmental monitoring activities. These include measurement of biodiversity and tracking of species. Many monitoring activities are carried out visually, which sometimes causes a lot of data errors. One way to overcome this problem is by using environmental DNA (eDNA). With eDNA, species can be detected using DNA scattered in the environment. *Hemiscyllium halmahera* is a new Indonesian endemic shark species discovered in 2013. The sharks inhabit mangroves, seagrass, and coral reefs. There have not been many observations regarding this endemic fish. The distribution and number of populations in their natural habitat is important to document, but because these sharks move at night, they are difficult to monitor visually. **Results:** The study was designed to detect this shark's distribution by eDNA. We strategically sampled their potential habitat at several islands of Halmahera. eDNA was filtered from 4 L of water at mid-water and sediment for each site using 20 and 0.4 μm filters. **Significance:** From these observational data, conservation activities can be designed, and we can reveal a broader understanding of walking shark (*Hemiscyllium halmahera*) distribution in Indonesia. The project will also increase our understanding of the use of eDNA and how it can be implemented in the conservation of endemic species in Indonesia.

Earthworm community diversity in the rainforest of French Guyana

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Background: It is well established that soil and its fauna support ecosystem services. Earthworms are abundant invertebrates which represent a substantial part of soil biomass. They are involved in essential ecological processes that govern soil function and the delivery of ecosystems services. Consequently, they have been recognized as a key component of the soil fauna. Despite their importance, they are not well researched, and their taxonomy is not yet fully understood. Studies of diversity patterns of earthworm communities and their genetic structure in tropical environments appeared only recently, and our investigation represents the latest contribution to this research by unravelling diversity patterns of earthworm communities along an environmental gradient in the French Guyana rainforest. **Results:** Earthworms were sampled for three habitat types (plateau – slope – swamp) at four localities of the French Guyana rainforest. We

generated 1286 COI sequences that were used to delimit operational taxonomic units (OTUs). In addition, several environmental variables were recorded at each sampling site and used to assess the level of diversity and structure for each earthworm community sampled. In particular, ANOVA and multivariate analysis were used to evaluate the statistical significance of environmental variables and the dissimilarity between sites and habitats, as well as β-diversity and rate of change (turnover) in species composition between sites. **Significance:** Our work, as well as previous studies at other localities, shows that the diversity of earthworm communities in the French Guyana rainforest was underestimated and that they are more spatially structured than initially thought. The application of DNA barcoding clearly helped to overcome the lack of taxonomic resolution that impeded earlier studies on earthworm communities. Our results represent a useful addition to the understanding of the diversity and structure of earthworm communities and the role environmental variables play in the formation of this structure.

Towards building a DNA barcode library of bird lice (Psocodea: Menoponidae, Philopteridae)

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Background: Bird lice (families Menoponidae and Philopteridae) are ectoparasites living in the plumage of birds. The lice spend their whole lifecycle on the body of their bird hosts, feeding on feathers and skin. Morphological identification of several taxa in these two families is challenging, due to their small size, different life stages, and morphological similarity. Therefore, DNA barcoding has a great potential to aid in identification and diversity studies of bird lice. No reference library of DNA barcodes is currently present for the species in the two families. In a new project "Bird lice in Norway", we aim to (*i*) build up new taxonomic expertise in collaboration with taxonomic experts, (*ii*) obtain DNA barcodes for bird lice species, and (*iii*) build a comprehensive reference collection for morphological identification. **Results:** Initially, we have mainly focused on shorebirds, in the avian order Charadriiformes. Our preliminary study on DNA barcoding of bird lice demonstrates that DNA barcoding works well for identifying and separating the species. So far, approximately 57 species of lice (= BINs in BOLD), from 45 species of bird hosts, have been barcoded. In addition to preliminary results, we also present a straightforward pipeline for building a DNA barcode reference library in parallel with a slide collection for morphological identification. **Significance:** We already see the potential of DNA barcoding as a strong tool to investigate cryptic species and new host–parasite interactions in bird lice. Building a DNA barcode reference library of this taxonomically challenging group will hopefully contribute to resolve issues which are difficult to investigate by morphological characters alone.

Investigating the community structure of eukaryotic benthic organisms by the extraction and analysis of limited mRNA

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Background: Marine benthic communities constitute an important part of the seafloor environment, as they are involved in a multitude of important ecological processes such as geochemical cycling and the distribution of pollutants. Studying the community structure and identifying its various constituents has therefore potentially important ecological applications. Although there are multiple methods that use DNA or rRNA as a source of taxonomic information and community structure analysis, only a few have attempted to do the same by using mRNA as the source of molecular material. We have therefore designed a robust workflow that can determine what organisms are present up to a reasonable taxonomic degree based on the mRNA present. **Results:** We assembled our mRNA sequences using Trinity assembly software. We compiled our own mitochondrial databases based on Barcode of Life Data Systems (BOLD), selecting for only the cytochrome c oxidase subunit I region. By sorting and filtering for the best sequences, we were able to detect groups of polychaete worms (Amphinomidae) and dinoflagellates that should be present within our samples, as they were also found in joint morphological studies. To verify the presence of the reported sequences, we corroborated our results by comparing them with the NCBI nr database after performing a reciprocal blast, by investigating the mapping properties of the sequences and by the construction and comparison of phylogenetic trees between the highest-ranking contigs. **Significance:** A successful workflow established by this project could facilitate and supplement future studies of ecosystem functioning as it can provide accurate and automated insights into the structural and functional responses of benthic communities. It also provides a unique application to a molecule that has had only limited use in community structure analysis before.

Ctenophores — native aliens in Norwegian waters

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Background: Ctenophores are reputedly defined as one of the most difficult groups of pelagic animals to work with—they are fragile and difficult to sample in good condition. A major challenge is that many fixatives used for preserving zooplankton cause distortion and dissolving of their gelatinous bodies, rendering the animals difficult or impossible to identify morphologically. At the same time, our taxonomical knowledge remains rudimentary at best. Consequently, this important component of the pelagic community remains neglected in most zooplankton studies and monitoring programs, and their diversity and ecological role are grossly oversimplified and misunderstood, leading to biased views of ecosystem functioning. Here, the biodiversity of ctenophores in Norwegian waters was described and documented, in several environments from the North Sea to the Arctic, by combining gentle sampling, a careful morphological examination of live specimens, and DNA barcoding. **Results:** Our results show that the ctenophore species richness has been underestimated and that a combination of morphological and molecular species identification methods is crucial. DNA barcoding allowed us to resolve diversity even in cases where species are superficially very similar, as is often the case with pelagic ctenophores. **Significance:** Our study facilitates an increase in taxonomic knowledge, which is a valuable first step towards establishing a baseline for future ecological studies, monitoring of climate impacts, and assessing the threat of introduced species. In addition, the development of a publicly available DNA barcode reference library has laid a solid foundation for future work on metabarcoding and environmental DNA (eDNA) applications for monitoring and ecosystem functioning.

Authentication of fishmeal by DNA metabarcoding

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Background: Fishmeal is a key component in a variety of animal feed, and the origin and composition is of importance for quality and market price. DNA metabarcoding can be used to identify the species and authenticate the content of fishmeal since commercially harvested fish species and potential by-catch are well represented in COI reference libraries. While DNA metabarcoding is of limited value in the identification of populations and origins within a species, it is well suited to identify and separate species. We tested the power of DNA metabarcoding for identification of species in mixed DNA samples of known fish species relevant to fishmeal production, and of a selection

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.

Barcoding the plants of mangrove ecosystems in China

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Background: Mangrove forests are essential ecological barriers, protecting people and their homelands in tropical and subtropical coastal regions. In China, mangrove forests are distributed along the coasts of provinces in South China. Due to the sharp loss of mangrove forest area in the past decades, proper management, conservation, and restoration of mangrove ecosystems, involving rapid identification of mangrove plant species using DNA barcoding, are in great demand. **Results:** We collected 559 plant specimens from mangrove forests of South China, covering all the morphological types, which were identified as 33 true mangrove species, 14 mangrove-associated species, and 22 nonmangrove species. Four candidate DNA barcodes (rbcL, matK, trnH–psbA, ITS2) were tested for the 559 specimens. As a result, 97.80%, 80.90%, 71.88%, and 63.81% of the specimens were successfully amplified and sequenced for rbcL, ITS2, trnH–psbA, and matK, respectively. At first, trnH–psbA was excluded from the list of barcode candidates for its high variation in SNPs and insertions-deletions. The statistics based on the genetic distance method indicated little barcode gap in the three candidate barcodes considered alone nor in the varied combinations of two markers. Evaluations using the similarity-based method indicated the combination of ITS2+rbcL had the highest correct score of Best Match/Best Close Match. The tree-based method indicated that ITS2+rbcL and ITS2 both showed the strongest discriminatory power. In summary, we suggest that ITS2+rbcL is the most promising potential barcode in mangrove plants. Using this barcode, we constructed a DNA barcode library for all the species collected and identified 17 hybrid individuals, which are morphologically hard to diagnosis. **Significance:** The barcode of ITS2+rbcL we tested, as well as the barcode library we constructed, will be useful in future mangrove conservation and restoration.

Alcohol is not the solution: different communities are recovered from storage ethanol and tissue homogenate of Malaise trap catches

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Background: Metabarcoding of the DNA extracted from preservative ethanol of insect bulk samples, instead of homogenized tissue, could bring a series of advantages such as reducing processing time and handling (reducing risk of cross-contamination). But, more importantly, it would allow researchers to conduct further work on intact insects (taxonomy, microbiome, diet, etc.) already barcoded. Previous studies have shown that analysis of preservative ethanol of freshwater benthos samples gives comparable results to analysis of homogenized tissue or morphological identification. However, these results do not necessarily apply to terrestrial insect communities because these differ in dominant life stages (larval/adult) and in the richness and abundance of different taxonomic groups compared to benthos samples. **Results:** We used two mitochondrial markers (COI and 16S) to metabarcode the ethanol-DNA and the homogenized tissue from the same samples of Malaise trap catches, and we found that the results from the two substrates were significantly different. We found that small and weakly sclerotized insect families were overrepresented in the ethanol substrate, and they were less probable to be detected in the tissue homogenate. Insect families with hard cuticles were, in turn, seldom detected in the ethanol, and the ones recovered in the tissue were in general large-bodied. **Significance:** According to our results, ethanol-DNA is not a good nondestructive alternative to tissue homogenization for terrestrial insect bulk samples. However, it could be used as a complement to the latter, as a way to increase the probabilities of detecting small and weakly sclerotized arthropods in a faster way than sorting by body size and processing the fractions separately.

DNA barcodes of basidiomycetes: large contribution from the Natural History Museum in Oslo

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Background: Within NorBOL (Norwegian Barcode of Life), the Natural History Museum in Oslo is responsible for coordinating the effort in building a DNA barcode library of Norwegian basidiomycetes. In this context, we have sequenced ~6000 fungal samples from specimens residing in the herbarium at the Natural History Museum. We used both freshly collected material and dried material of up to 15 years of age. **Results:** The sequencing success rate was 90%. Neither the sampling of fresh versus dried material, nor what order or family the species belonged to, affected the level of sequencing success, but success decreased with age of material. The 5300 successful sequences comprise more than 2000 species, and so far (January 2019), more than 1700 species are validated and made public in BOLD. This means that 44% of the Norwegian basidiomycetes are DNA barcoded with high confidence in the correctness of the species identifications. **Significance:** Fungal species are difficult to identify because of the existence of cryptic species and hidden diversity in most groups, and there is still a large proportion of species to be discovered. A DNA barcode library of fungi is therefore of great importance. In addition to having contributed the most basidiomycete sequences to BOLD (besides GenBank), our project has validated and improved the species identifications in our collection, as well as facilitated new knowledge in fungal taxonomy.

OLICH: a reference library of DNA barcodes for Nordic lichens

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Background: Lichens are fungi living in mutualistic symbiosis with algae, and the lichen taxonomy and nomenclature follows that of the fungal exhabitant. There are currently recognized more than 19 000 species in the world, i.e., about 17% of all known fungal species and 27% of all known ascomycete species. In the Nordic countries, ~2500 species are recognized. Identification of lichen species has traditionally been based on morphological, anatomical, and chemical characters, but DNA-based identification is now increasingly being used. **Results:** We here present a DNA barcode dataset of ITS sequences from 506 lichen species occurring in the Nordic countries, represented by 1321 sequences. **Significance:** Analyses of the dataset reveal interesting new insight into several genera where traditional methods have not identified critical species, e.g., *Cetrelia*, *Nephroma*, *Peltigera*, *Psoroma*, *Ramalina*, and *Sticta*.

What shapes bat's diets? Trophic snapshot of a Mediterranean bat community

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Background: Predator–prey interactions forge the behaviour and ecology of all organisms and are therefore crucial in structuring ecological communities. In bats, these are believed to be constrained by the interaction of species morphology, echolocation, and foraging behaviour. Several studies have emphasized the role of these traits in prey acquisition and have tried to group bats accordingly in guilds. However, due to practical difficulties in studying entire communities

and the constraints of morphology-based diet analysis, it is not clear how these guilds actually correlate to the diet of insectivorous bats. To better understand this question, we used DNA metabarcoding to assess the diet of a Mediterranean bat community located in northeast Portugal composed of 19 different species. **Results:** Bat droppings were collected from 486 individual bats, and DNA was extracted from 1206 individual pellets (up to three per bat). We amplified prey DNA using two nonoverlapping COI markers, ZBJ and Fwh2, to detect the maximum number of prey at the highest taxonomic resolution possible. We used a canonical and ecological network analysis to assess the dietary guilds. Bats were structured in 4–5 diet groups, three of which were very distinct and fed on either noctuid moths, crickets, or spiders, respectively. The fourth and fifth groups were mainly characterized by not feeding on these taxa and ingesting more Coleoptera, Diptera, and Hemiptera. None of the diet groups corresponded to previously suggested echolocation or foraging guilds, with the exception of *Myotis myotis*, the only ground-gleaning bat in our community, which formed a guild on its own. **Significance:** For the first time, we provide empirical evidence for the existence of dietary guilds in Mediterranean insectivorous bat communities and show that these do not correlate to previously proposed guilds based on echolocation signal and foraging habitat, mode, site, or vegetation clutter.

Influence of accuracy, repeatability, and detection probability in the reliability of species-specific eDNA-based approaches

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Background: Environmental DNA (eDNA) barcoding has a high potential to increase the cost-efficiency of species detection and monitoring in aquatic habitats. However, despite vast developments in the field, many published assays often lack detailed validation, and there is little to no (commonly agreed upon) standardization of protocols. **Results:** In this study, we evaluated the reliability of eDNA detection and quantification using published primers and assays targeting the Freshwater Pearl Mussel as a model organism. We first assessed limits of detection for two different target genes (COI and 16S) following the MIQE guidelines, and then tested the reliability of quantification in a double-blind mesocosm experiment. Our results reveal that different methodological indicators, namely accuracy, repeatability, and detection probability affected the reliability of eDNA measurement at the different levels tested. The selection of the optimal analytical method was mainly determined by detection probability. Both the COI and 16S assays were highly specific for the targeted organism and showed similar accuracy and repeatability, whilst the limit of detection was clearly lower for the COI-based approach. In contrast, the reliability of eDNA quantification hinged on repeatability, reflected by the scattering (R^2 = 0.87) around the relationship between eDNA and mussel density in mesocosms. A bootstrapping approach, which allowed for the assignment of measures associated with repeatability of samples, revealed that variability between natural replicates (i.e., accuracy) strongly influenced the number of replicates required for a reliable species detection and quantification in the field.

The effects of genetic coverage on phylogenetic imputation performance in life history trait datasets

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Background: Missing observations in trait datasets pose an obstacle for analyses in the fields of ecology and biodiversity science. Imputation techniques offer an alternative to removing cases with missing values from datasets and can contribute to trait-based analyses, as novel taxa are frequently discovered during high-throughput biodiversity surveys. In particular, imputation techniques that incorporate phylogenetic information into their estimations improve accuracy over standard techniques. Phylogenetic information is usually included in the form of a multigene tree; however, it remains to be explored whether the amount and type of genetic data included in the tree affects imputation accuracy. Our objective is to perform a large-scale comparison of selected imputation methods that considers a variety of data types, taxa, and different genes (including standard DNA barcode markers) as the source of phylogenetic information. **Results:** Continuous and categorical trait data were mined from databases including BOLD, FishBase, and PanTHERIA. Prior to imputation, sequence data for several mitochondrial and nuclear markers were assembled and gene trees constructed. Known data were removed at varying levels from complete datasets (e.g., 10%, 20%, 30%, etc.) and imputation techniques applied to each dataset to fill in the best estimate of the missing value. Methods include k-nearest neighbor (kNN), phylogenetic eigenvector regression, random forest, and multiple imputation chained equation (MICE). The extent to which each gene tree improves imputation accuracy is being quantified, and preliminary results are promising in terms of assessing imputation performance. **Significance:** This comparison of imputation techniques and the phylogenetic resolution provided by specific genes in the imputation process may be used to develop strategies to overcome the loss of sample size due to missing data in trait analyses. Finally, insights may be obtained regarding trait associations with evolutionary rates or specific variants in particular genomic regions, such as COI.

DNA metabarcoding of plant–soil micro- and macrobiomes for terrestrial restoration

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Background: Simultaneous assessment of the above- and belowground biotic components are of particular interest as land-use changes and individual tree species are likely to have a direct effect on the composition of surrounding soil biotic communities. Tree species-generated microbial and invertebrate heterogeneity in soil might be an important factor in facilitating regeneration as recovering ecosystems often contain tree community composition reflecting previous land-use legacies. Determining the importance of plant–species effects on belowground biotic communities in tropical forests is essential for predicting current and future biodiversity. However, considerable effort is needed to include and study the interactions of soil invertebrates across land-use and with individual plant species for restoration. Here, we provide evidence via DNA metabarcoding that soil microbial and invertebrate communities were distinct between two tree species (*Dipteryx panamensis* and *Pentaclethra macroloba*) in Costa Rica. **Results:** Soil $NH4^+$, $NO3^-$, and microbial biomass C, and bacterial, fungal, and invertebrate soil community composition were significantly different between the immediate surrounding soils of the two tree species ($p < 0.05$). Out of the soil variables assessed, there was a strong association of soil $NH4^+$ shaping soil bacterial, fungal, and invertebrate community composition ($p < 0.05$). In addition, percent dissimilarity increased moving from bacteria, to fungi, to invertebrate community composition, suggesting different trophic levels are affected at different magnitudes. **Significance:** This is the first study in the region to weave the influence of plant–species specificity on not just soil bacterial and fungal communities, but also invertebrates. These findings provide an avenue via DNA metabarcoding for future assessment of conservation efforts that facilitate plant–species reintroduction programs.

DNA barcoding for forensics: experiences from three years of BopCo

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Background: The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo) aims to act as a focal point for the species identification of biological materials of policy concern in Belgium and beyond. This identification service, be it by morphology or DNA barcoding, is available to any stakeholder (e.g., forensic investigators, insurance brokers, legal services) who deals with biological materials of policy concern and is in need of an accurate identification. Additionally, cases of interest often rely on reliable reference sequence databases, where BopCo also plays an important role. In this talk, we present examples of forensic identification requests handled by BopCo. **Results:** Among other examples that will be discussed are (*i*) the identification of species involved in birdstrikes based on feathers, blood, and(or) tissue remains, in order that civilian and military flight management can implement appropriate strategies; (*ii*) the construction of reference DNA-barcode libraries for the Belgian forensic rove beetles (Staphylinidae) and fly species (Calliphoridae, Muscidae, Fanniidae) in La Reunion, whose species-specific developmental timing is informative for crime investigators to estimate the post-mortem interval; (*iii*) several CITES-related requests, e.g., the identification of a bivalve shell used in a piece of art intercepted at the airport and the screening of confiscated dietary pills for the presence of a CITES-listed *Aloe* species; and (*iv*) requests to identify domesticated dogs and cows to the level of breed using hair samples. **Significance:** Forensic samples often comprise only pieces or fragments of organisms, yet still need accurate identification so that suitable actions can be taken and(or) rules and policies implemented. In these cases, DNA barcoding offers an important added value to perform reliable species identifications. In our experiences, however, we have also encountered limitations of the technique; this emphasizes the importance of an integrated approach when handling difficult samples.

DNA barcoding to identify invasive alien species targeted by EU policies

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Background: The introduction of invasive alien species (IAS), whether by accident or deliberately, can have serious negative consequences in their new territory when they manage to establish viable populations. Their presence can disrupt natural and managed ecosystems, leading to biodiversity loss; affect crops and livestock; and might introduce vector-borne diseases or parasites, impacting human health. In order to protect the European native biodiversity and ecosystems, and to mitigate the potential impact on human health and socio-economic activities, the issue of IAS is addressed in EU Regulation 1143/2014. The IAS Regulation currently gives priority to 49 species and is enforced across all member states. In order to implement the proposed actions, however, methods for accurate species identification are required when suspicious biological material is encountered. Because morphology-based species identifications are not always possible (e.g., cryptic species, trace material, early life-stages), the Barcoding Facility of Organisms and Tissues of Policy Concern (BopCo) investigated and evaluated the usefulness of DNA sequences to identify each of the 49 IAS of EU Concern. **Results:** For each IAS in the EU regulation, BopCo produced a factsheet, giving identification advice based on the publicly available DNA sequence data and various other sources. Each factsheet contains information on species taxonomy and current distribution in Europe, as well as a discussion on the usefulness of publicly available DNA sequences to identify samples to the taxonomic level stated in the EU list. Issues preventing a reliable identification are defined and discussed too. **Significance:** This project aims to provide an evaluation of the usefulness of DNA sequence data available for accurate identification of the 49 IAS of Union Concern, as well as identify the data gaps in the DNA reference databases. A future perspective to the project is to then fill these gaps, where possible, with new sequence data.

SCANDNAnet — validating and intercalibrating metabarcoding for routine use in Nordic freshwater biomonitoring

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Background: DNA metabarcoding holds great capacity for the assessment of macroinvertebrates in aquatic ecosystems. However, few large-scale studies have compared the performance of DNA metabarcoding with that of routine morphological identification. We will metabarcode several hundred macroinvertebrate samples from stream and lake sites across Denmark, Finland, Iceland, Norway, and Sweden. The samples were collected in 2017 and 2018 using national sampling protocols. Specimens were identified by morphology, following standardised protocols used in the routine national monitoring programs. **Results:** Our preliminary results presented at the conference will likely show that DNA metabarcoding identifies more than twice the number of taxa compared with the morphology-based protocol, and yield a higher taxonomic resolution. We will calculate and compare the ecological status assessment metrics from morphological and DNA metabarcoding datasets. **Significance:** We show that DNA metabarcoding is applicable in routine national monitoring programs at large scale. DNA metabarcoding represents a feasible and reliable method to identify macroinvertebrates in aquatic bioassessments and offers powerful advantages over morphological identification in providing identification for taxonomic groups that are unfeasible to identify in routine protocols.

Development of eDNA metabarcoding markers for Neotropical fish species based on the mitochondrial 12S region

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Background: The mega diversity found in the Neotropics represents a challenge for species detection and monitoring using noninvasive methods such as environmental DNA (i.e., DNA extracted from environmental samples). Challenges range from the lack of a comprehensive reference DNA database for taxonomic assignment to standardized methods for environmental DNA (eDNA) detection and molecular markers able to detect the entire biodiversity. Here, we built a reference database using the 12S mitochondrial region and developed and tested primers (<200 bp) for eDNA analyses using 67 fish species (70 molecular operational taxonomic units (MOTUs)), representing 54 genera, 25 families,

and six orders from the São Francisco River Basin (southeastern Brazil). **Results:** We obtained 132 DNA sequences from the 12S region (565 bp) and used it as a reference for developing new primers sets targeting a 193 bp fragment. To test amplification efficiency of the primers, we applied in silico and in vitro approaches. In vitro tests using tissue samples demonstrated an efficient amplification for all species analyzed and also for eDNA retrieved from water samples from an aquarium containing *Geophagus brasiliensis*. To evaluate the efficacy of the 12S mini-barcode region in delimiting species, we conducted four species delimitation analyses based on Bayesian approaches (GMYC, bPTP) and genetic distances (ABGD, Tamura-Nei). The Bayesian analyses, GMYC and bPTP, identified 70 and 76 MOTUs, respectively. Distance-based analyses, ABGD and Tamura-Nei, recovered 62 and 72 MOTUs, respectively. Thus, GMYC was accurate in pointing out the 70 MOTUs previously identified within the 67 morphospecies. In silico PCR did not detect nontargeted organisms' amplification such as arthropod, bacteria, mollusks, or mammalian (including *Homo sapiens*). **Significance:** In silico analyses demonstrated that the fragment analyzed contains enough resolution to differentiate all 67 species and may be useful for an ecoregion-scale eDNA metabarcoding biodiversity evaluation, helping with the complex task of monitoring and conserving the Neotropical ichthyofauna.

The key to the treasure chest — selections of herbarium specimens for DNA extraction

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Background: There are several billion plant specimens laying in the world's herbaria, and they hold great opportunities for genetic studies and the creation of reference libraries. However, the quality of the extracted DNA may vary with the initial DNA quality as a result of when the specimen was collected, drying conditions, and storage time. This also affects the amount of endogenous fungi present on the specimens. Here, we share our experience on how we selected samples, performed DNA extraction, shotgun sequenced, and assembled the DNA sequences of 2000 specimens from Norway and the polar regions, mainly from the herbarium collections at the Tromsø Museum. **Results:** Preliminary results indicate that age of the specimens is not an important factor for obtaining good sequence data. However, factors such as parasitic infections and the time of the year the specimen was collected in the field seems to have an impact. Further analysis of factors, e.g., amount of sample (mg dried weight), is ongoing. **Significance:** By selecting specimens from the world's herbaria that are most suited for a successful genetic pipeline, we hold the key to a huge treasure regarding genetic studies. By selecting specimens with favorable criteria, we get a higher success rate regarding obtaining DNA sequences from herbarium material, which again gives lower cost regarding re-extractions and lowers the labour force required.

Enhancement of Croatian forest ecosystem services through assessment of fungal diversity based on DNA barcoding (ForFungiDNA) project

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In Europe, Croatia is distinguished by great biodiversity due to its position on the dividing line between Continental, Mediterranean, Pannonian, and Alpine biogeographical regions. About 47% of the total land area of the Republic of Croatia is covered in forests. These are the most complex terrestrial ecosystems and contain a large number of fungal species. Fungi play key ecological roles in forest ecosystems and are indispensable for forest health and existence. Human overexploitation of natural resources, and the geographical position of Croatia in the hot-spot area for climate change, poses a great threat to the biodiversity of its forests. Fungi are by far the least-studied group of organisms in Croatia with only 25% of expected species recorded so far. The main aim of this four-year project (2018–2022), funded by the Croatian Science Foundation, is to study fungal biodiversity of Croatian forests through the DNA barcoding methods and to analyze its impact on forest ecosystem services. The goal of ForFungiDNA project is to add at least 1500 fungal DNA barcode sequences (ITS rDNA) to international bioinformatic databases (GenBank, BOLD). It is planned, as a result of fieldwork research, to collect and deposit at least 1000 new fungal samples in the Croatian National Fungarium (CNF). Finally, all species DNA barcoded in this project will be categorised in trophic groups (pathogens, saprotrophs, and mycorrhizal species), and the intensity of their impact on the forest ecosystem services will be assessed. The project results will have great potential for different applications in forestry, food industry, pharmacy, and nature conservation. Application of DNA fungal barcodes will be especially important in forestry for relatively fast and accurate identification, control, and suppression of plant pathogens, as well as for identification of mycorrhizal and saprotrophic species.

Spatial variation in Aotearoa (New Zealand) groundwater Crustacea

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Background: This project aims to define the biodiversity of New Zealand groundwater ecosystems and natural variability contained therein. Specimens of amphipod, isopod, and copepod crustaceans were collected from sampling wells in the North and South islands of Aotearoa (New Zealand). **Results:** A total of 280 specimens was collected, and, of these, 149 were successfully sequenced for COI (53% success rate). A total of 44 BINS was obtained with high levels of endemism and diversity found for individual wells. Several putative species (e.g., *Paraleptamphopus subterraneus*) were found to be complexes of genetically distinct individuals, each unique to a specific location. Patterns of land-use activities (e.g., agriculture) did not seem to influence faunal composition. **Significance:** We conclude that geological isolation rather than land-use patterns are most likely to explain the patterns of diversity we observed. The reference library we have begun assembling will provide a baseline for assessing groundwater ecosystem biodiversity and ecosystem health as well as to deliver a foundation for next-generation sequencing (environmental DNA (eDNA)) approaches to measuring and monitoring these ecosystems.

Dual DNA barcoding and metagenomics as basis of precision-based diagnosis of mycoses

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Correct and fast identification of the agents of mycoses is of great importance to enable early diagnosis and targeted antifungal therapy. DNA barcoding offers an accurate, fast, cost-effective, culture-independent approach for species identification. In 2015, the first quality-controlled primary fungal DNA barcode (internal transcribed spacer (ITS1/2) region) database for human and animal pathogenic fungi, the ISHAM-ITS database, was established, currently containing 4200 ITS sequences from 645 species. With the ITS1/2 region only being able to correctly identify 75% of all fungi, the translation elongation factor 1α (*TEF1α*) was introduced as the secondary DNA barcode locus, containing 909 *TEF1α* sequences from 186 species. We are cur-

rently in the process of annotating 4865 *TEF1α* for addition to the now-combined ISHAM DNA barcoding database. To assess the resolution power of the ITS1/2 and *TEF1α* loci, the inter- to intraspecies genetic distance within taxa sharing the same phylogenetic lineage was compared. *TEF1α* showed less intraspecies and higher discriminatory power at the interspecies level than the ITS1/2. The introduction of Next-Generation Sequencing (NGS) (e.g., the MinION™ from Oxford Nanopore Technologies, a palm-sized sequencer with low initial start-up costs) allows now for a high-throughput, real-time, long-read (average 10–15 kb) simultaneous identification of complex samples (metabarcoding). To assess the ability of genome-wide, long-read, metagenomic sequencing, we used the MinION™ to identify *P. jirovecii* directly from respiratory-tract specimens and to characterise the associated mycobiome. *P. jirovecii* DNA was detected in bronchoalveolar lavage (BAL) and induced sputum (IS) samples from three patients with confirmed PCP. Other fungi present in the associated microbiome included known human pathogens (*Aspergillus*, *Cryptococcus*, *Pichia*) as well as commensal species (*Candida*, *Malassezia*, *Bipolaris*). Our results indicate that false-positive and error-prone reads currently represent a real challenge for metabarcoding studies. To overcome these issues, more accurate taxonomy assignment algorithms and reference databases are needed.

Using DNA barcoding to protect Aotearoa (New Zealand) native flowering plants

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Background: Aotearoa (New Zealand) is a biodiversity hotspot, and most of our native plants are found nowhere else in the world. Our native flowering flora is relatively small (~2000 species), and hence highly tractable. The last comprehensive treatment of our botanical diversity was >50 years ago, leaving a vast gap in contemporary knowledge of our flora. Additionally, numerous native plants are utilised by tohunga (indigenous healers), and the documentation and conservation of these taonga species (treasurers) is of the utmost importance. **Results:** In total, 385 specimens, a mixture of freshly collected and herbaria samples, were sequenced for four common plant barcoding markers; matK, rbcL, ITS, and psbA–trnH; at a high-throughput facility. Additional specimens were sequenced in-house, in small batches. Sequencing success varied across all markers but consistently showed that fresh samples produced significantly better results across a broad spectrum of plants than dried herbarium specimens, especially when considering high-throughput samples. ITS was the most informative marker for species delimitation. **Significance:** The results will guide future efforts and reinforce that while herbarium samples are valuable, for some taxa fresh materials will be required. We obtained many of our fresh materials from botanical gardens, which highlights the utility of these valuable resources. Protection of Aotearoa's tāonga is of utmost importance; one thread of our work is tied to mātauranga Māori (indigenous knowledge) to provide a permanent means to accurately document these plants. Our project serves as the model for further groups such as weeds, which are often difficult to identify and having few taxonomic experts, with biosecurity groups/organisations the targeted end-users.

Inextricably linked: revealing the connections between neighbouring communities through DNA metabarcoding of host–parasitoid networks

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Background: Indirect effects such as apparent competition have been proposed as important factors in the structuring of arthropod communities. Molecular characterisation of trophic interactions via DNA metabarcoding allows the reconstruction of ecological networks, thus tracing the pathways for direct and indirect effects. Apparent competition can also reveal whether spatially separated insect herbivore populations are interconnected via shared predators or parasitoids. This is of particular interest in agricultural landscapes, where natural and semi-natural habitats are intermingled with intensively managed cropland, leading to large variations in insect herbivore densities over short distances, and consequently to indirect effects between communities in juxtaposed habitats. We use DNA metabarcoding to build 22 highly resolved host–parasitoid networks within adjacent crop and noncrop habitats nested within an agricultural intensification gradient. We then use these networks to assess how apparent competition manifests within and between these habitats. **Results:** Arthropod communities in highly productive crop habitats exert a strong influence on communities in surrounding semi-natural habitats, with effects mediated by shared parasitoid species. This effect appears to be partially governed by the management of surrounding landscapes, such that the potential for apparent competition is reduced in areas of high agricultural intensification. Findings may stem from landscape-mediated variation in pest or parasitoid density, species richness, or community structure. **Significance:** Agricultural landscapes are patchworks of crops and noncrop habitats. Since crops form patches of highly productive habitat in a less-productive matrix, we expect significant flows of biomass across the landscape. A sound description of network-level patterns, processes, and outcomes is a key prerequisite for (*i*) understanding how natural communities are linked in the real world, (*ii*) understanding spatiotemporal patterns of pest damage, and (*iii*) designing landscapes for efficient biological control. DNA metabarcoding provides a technical breakthrough for advancing these endeavours.

A new tool to identify the target 16S rRNA sequence from next-generation sequencing data

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Background: The development of high-throughput sequencing methods has revolutionized our understanding of microbial community composition. A common approach in amplicon sequencing reads is clustering sequences into operational taxonomic units (OTU) based on a percentage sequence similarity threshold. However, it has been shown that sequence abundance plays an important role in OTU inference. There are also many criticisms of using percentage sequence similarity to define OTUs. Most of all, percentage sequence similarity is a nonevolutionary based distance metric. Moreover, hierarchical clustering methods can overestimate the evolutionary similarity between pairs of sequences, and only a single sequence is selected as a representative for OTU identification. We focused on selected targeted *Wolbachia* endosymbiont sequences of tardigrade host species from NGS datasets, using OTUs as reference sequences and sequence similarity defined based on p-distance values. **Results:** Each of the selected sequences was locally aligned, using Biopython's pairwise2.align.localxx (+1 for identity, 0 for mismatch, 0 for gap) implementation of the dynamic programming local alignment algorithm. Then, for each aligned pair of sequences, the alignment score was reported and p-distance was calculated (0 for match, 1 for mismatch, gaps as mismatches). The p-distance for all 16S rRNA sequences that were identified as *Wolbachia* endosymbiont did not exceed 0.07%. Subsequently, similarity between sequences was assessed using a BLAST search (high identity ≥98% with a query coverage approximating 100% and an E-value near 0.0 were accepted). **Significance:** In the era of next-generation sequencing data methodologies, there is still a need for a

systematic and intelligent approach in order to be able to process obtained data in an efficient way. We present an analysis based on targeted sequences of one bacterial species, but it can be easily performed for any other OTU comparison. Finally, this study opens up new possibilities for analyzing the diversity of the microbiome.

Molecular evidence for *Wolbachia pipientis* Hertig, 1936 endosymbiosis in tardigrades opens new research opportunities for DNA barcoding

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Background: Tardigrada consists of ~1300 species. Due to their ability to enter the state of cryptobiosis, they are able to inhabit and survive in extreme terrestrial and aquatic environments. Bacterial endosymbionts exert a significant impact on the microevolution and reproductive ecology of their hosts in terms of parthenogenesis induction, female-biased sex ratios through feminization, male killing, and cytoplasmic incompatibility. Interestingly, they can also shape patterns of host mitochondrial genetic diversity by linking infection patterns with phylogenetic clades as well as certain haplotypes. Such infections have been detected in many metazoans. Until now, the hypothesis that *Wolbachia* or other bacterial endosymbionts may be present in tardigrades has remained almost unexplored. **Results:** In order to identify tardigrade endosymbionts and bacterial communities, high-throughput sequencing of the 16S rRNA bacterial gene fragment was conducted, which resulted in a total of 83 275 reads. Identified operational taxonomic units (OTUs) were classified as Proteobacteria (66.90%), Bacteroidetes (23.53%), Firmicutes (5.21%), Cyanobacteria (2.99%), Verrucomicrobia (0.59%), Actinobacteria (0.52%), unclassified at Phylum level (0.21%), and Chloroflexi (0.01%). Finally, we have identified *Wolbachia pipientis* (0.0014%), a bacterial endosymbiont belonging to the order Rickettsiales. **Significance:** The discovery of endosymbiotic infections of *Wolbachia pipientis* in tardigrades opens new opportunities in tardigrade studies focused on, for example, coevolution. This bacterium is mainly maternally transmitted and can contribute to the loss of species mitochondrial genetic diversity through selective sweeps. In addition, it is associated with a reduction in effective population size, which in turn may lead to lower mitochondrial diversity. Moreover, mitochondria are derived from α-proteobacteria, and thus sequence similarities can be observed not only in the mitochondrial barcode genes, but also NUMTs and nuclear mitochondrial DNA segments. This may lead to erroneous DNA barcoding and species misidentification. Lastly, we hypothesize that parthenogenetic reproduction in tardigrades may be induced by *Wolbachia pipientis* infection.

Molecular systematics and species delimitation in *Coniocarpon* and *Arthonia punctiformis* sensu lato (Arthoniaceae) in Norway

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Background: The diversity and distribution of relevant groups of crustose lichens in Norway are incompletely known due to insufficient collections and the presence of morphologically similar but genetically distinct species. This applies, for example, to *Coniocarpon* and *Arthonia punctiformis* s.lat. in Arthoniaceae. Two species of *Coniocarpon* are known from Norway, *Coniocarpon cinnabarinum* and *Coniocarpon fallax*. Both have their main distribution in the country in the boreonemoral rainforests. The distinction between *C. cinnabarinum* and *C. fallax* has been problematic due to the ambiguous interpretation of morphological characters. I will study the species delimitation and distribution of *Coniocarpon* in Norway based on morphological and molecular data. *Arthonia punctiformis* is widespread in Norway in coastal to alpine habitats. Preliminary molecular data indicate that the species is heterogeneous, both in Norway and at the world level. I will investigate whether or not *A. punctiformis* is a polyphyletic taxon. **Results:** A phylogeny of *Coniocarpon* based on Bayesian and maximum likelihood analysis of the mtSSU, nLSU, nITS, and RPB2 gene regions show three genetically distinct lineages representing *C. cinnabarinum*, *C. fallax*, and *Coniocarpon* spec. The molecular results are supported by morphological characters, such as differences in ascoma morphology, ascospore size, ascospore septation, and distribution of the pruina. A phylogeny of *A. punctiformis* based on Bayesian and maximum likelihood for the mtSSU, nLSU, ITS2, and RPB2 gene regions show that specimens collected from *Corylus avellana*, *Hippophae rhamnoides*, *Sorbus aucuparia*, and *Tilia cordata* differ from those collected from *Betula nana*, *Betula pendula*, and *Betula pubescens*. Morphological differences have not yet been discovered between the two genotypes. **Significance:** Increased knowledge in insufficiently known groups such as *Coniocarpon* and *A. punctiformis* s.lat. are important for improved natural resource management and biodiversity conservation in Norway.

A barcode reference library of stream diatoms from Central Mexico: setting the baseline for eDNA-based diversity assessments and biomonitoring

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Background: Diatoms are the most diverse group of algae and one of the most commonly used bioindicators. But despite the numerous advantages of using diatoms as bioindicators, their study in Mexico combining morphological with molecular approaches is scarce. In order to set an identification baseline for future environmental DNA (eDNA)-based diversity assessments and biomonitoring, this study presents a barcode reference library of stream diatoms from Central Mexico, using nuclear (18S V4 rDNA) and plastid (rbcL) markers. Furthermore, the 18S V4 library was used as a reference database in an eDNA metabarcoding study to assess diversity, comparing its results with a morphological assessment. **Results:** A total of 300 clonal non-axenic strains was established, belonging to 110 infrageneric taxa. Each strain is documented with (*i*) voucher and DNA Bank deposition number; (*ii*) sampling locality, date, collector, and isolator; (*iii*) environmental data from sampling locations; (*iv*) light microscopy images, in some cases also from scanning electron microscopy; (*v*) morphometrics; and (*vi*) references to taxonomic identification sources. The retrieval of barcodes was higher for rbcL (95%, 290 sequences) than for 18S V4 (65%, 195 sequences). Regarding species resolution, rbcL was more effective in discriminating among closely related species than 18S V4. The metabarcoding study showed a larger diversity than the morphological one; however, by combining both methods, an even larger diversity was detected. **Significance:** This is the most comprehensive taxonomically curated barcode reference library produced for stream diatoms from Mexico, setting the baseline for future eDNA diversity assessments and biomonitoring in this region located within two world biodiversity hotspots. Our combined diversity assessment (metabarcoding and morphology) highlights the complementarity of classical taxonomy and eDNA metabarcoding. Each barcode sequence is linked to museum-deposited vouchered material (available via, e.g., INSDC, GGBN, GBIF, PhycoBank), allowing data traceability back to the original specimens.

A DNA barcode library for 50% of Diptera reported for Germany and its implications for biodiversity monitoring

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In this study we present the results of the DNA barcoding campaign focussing on German Diptera. The DNA barcode library includes more than 45 000 specimen records for approximately half of the fauna reported for Germany—it comprises almost 2500 species with a full taxonomic assignment and another 2700 unnamed BINs, so-called dark taxa belonging to 88 families. Until now, most of these families, especially the most diverse ones, have been taxonomically inaccessible because of the lack of specialists. By contrast, within a few years this study provided an interim taxonomic system for half of the German Dipteran fauna, which will provide a useful foundation for subsequent detailed, integrative taxonomic studies. To demonstrate the use of this reference library as an effective method for biodiversity studies and for species delineation using BINs and operational taxonomic units (OTUs) using DNA metabarcoding, we used DNA extracts derived from bulk collections made by Malaise traps. As the reference libraries will continue to grow, and gaps in the species catalogue will be filled, BIN lists assembled by metabarcoding will gain incremental taxonomic resolution. Here, we present (*i*) a DNA barcode library for 5200 species (BINs) of Diptera; (*ii*) that DNA barcode clusters, labelled with globally unique identifiers (such as OTUs and(or) BINs), by the example of bulk extractions from a Malaise Trap experiment, provide a pragmatic, accurate solution to the taxonomic impediment; and (*iii*) that assigning interim names based on BINs and OTUs obtained through metabarcoding is an effective method for studies on species-rich groups that are usually neglected in biodiversity research projects because of their unresolved taxonomy.

Barcoding two imaginary insect species

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Background: Although DNA barcoding has proven to be an efficient tool to distinguish species, cases of failure are regularly reported as well. In such instances, typically two, but sometimes more, species either show close similarity in barcodes and(or) do not form monophyletic clusters in a phylogenetic tree. Barcode sharing among species may result from true evolutionary processes, particularly introgression and incomplete lineage sorting, but also from operational factors, such as misidentifications, undetected cryptic diversity, and oversplitting of species. Recent studies suggest that operational factors may explain a significant proportion of cases of failure of DNA barcoding. **Results:** We studied two cases of full barcode sharing, a noctuid moth *Mesapamea remmi* (being identical with *M. secalis* and nearly identical with *M. didyma*) and a true bug *Psallus wagneri* (being identical with *P. perrisi*) using a genome-wide ddRAD sequencing method. Extensive data sets of ~9400 loci/27 539 SNPs for *Mesapamea* and ~19 000 loci/195 414 SNPs for *Psallus* did not reveal a single diagnostic site of *M. remmi* and *P. wagneri*, and in both cases, species were also fully intermixed in phylogenetic trees. We conclude that neither *M. remmi* nor *P. wagneri* represents a real species. *Mesapamea remmi* shows abnormal genital features suggesting its reproductive sterility. Males of *P. wagneri* are easily told apart from those of *P. perrisi* by genital structure, whereas females are indistinguishable. Based also on patterns of occurrence, we conclude that *P. perrisi* represents a species showing genital polymorphism. **Significance:** The two cases analysed here demonstrate that DNA barcode sharing should always be studied for operational factors. Because such factors appear to be common, the functionality of DNA barcoding is likely higher than suggested in most studies. Furthermore, the studied cases demonstrate that genital traits are not always invariable, but may show intraspecific polymorphism like other structures affected by sexual selection.

Low genetic diversity in *Triops granarius* (Crustacea, Notostraca) from vernal pools in Qatar

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Background: Habitat loss is a real threat in rapidly developing countries such as Qatar, where the fauna and flora may not have been described before species are permanently lost. The recent discovery of the tadpole shrimp *Triops granarius* in vernal pools in northern Qatar calls for such investigations. Samples were collected in winter of 2017–2018, and the DNA barcoding gene COI was used to analyze 12 samples from the northern pool and 16 samples from the southern pool, respectively. **Results:** Overall, the sequences showed high between- and within-population uniformity, in which the southern pool displayed polymorphism at only two nucleotide positions, while the northern pool exhibited polymorphism at only one, different position than in the individuals from the southern pool. The six total haplotypes yielded mean divergence within each pool at about 0.31% and 0.15%, respectively, well below the suggested threshold of 3% for within-species divergence in the barcoding gene. The intraspecific phylogenetic analysis with other published sequences in GenBank suggests a closer relationship with samples collected from Mongolia and Japan, with a sequence divergence of 8%, while more distant compared to those from India and five northern and southern African countries. **Significance:** The organism is the largest natural freshwater species in Qatar, where no standing freshwater exists. The high divergence level between the populations in Qatar and those from other geographic regions suggests the need to re-examine the species status of this most widely distributed species of the genus.

DNA barcodes and beyond: using targeted capture to distinguish bamboos and understand giant panda diet

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Background: Giant pandas (*Ailuropoda melanoleuca*) mainly eat bamboo. However, beneath this simplistic statement lies a complex issue—bamboo species are difficult to tell apart morphologically, and the taxonomic resolution in most DNA barcodes is limited. Over 60 bamboos have been reported in giant panda diet and(or) habitat. In addition, pandas occasionally eat food other than bamboo. To develop a detailed understanding of a dietary specialist, we tested a targeted capture (or Hybseq) approach to obtain genetic data from giant panda faecal samples for both standard DNA barcodes and a set of bamboo nuclear genes. **Results:** A set of diagnostic SNPs and short gene regions were identified from the analysis of >30 bamboo species sam-

pled throughout giant panda habitat. A targeted capture approach, combining nuclear and plastid genes, including standard DNA barcodes (e.g., ITS, trnL, rbcL, matK), was successfully used to obtain genetic sequence data from wild giant panda faecal material. Genetic sequence data was obtained for over half the bamboo nuclear genes investigated. Furthermore, we obtained data from multiple DNA barcodes representing a range of taxa, including giant panda, bamboos, nonbamboo plants, invertebrates, and fungi (some which likely represent environmental contamination). **Significance:** Targeted capture can provide information from multiple markers and in degraded samples, where fragments are short. We show this method can be applied to faecal samples to investigate diet, simultaneously providing information on multiple DNA barcodes as well as information from nonstandard nuclear genes, which can provide additional data to achieve species-level resolution. Furthermore, it may also be used to obtain information on the source of the samples, including the species, sex, and individualisation. This greatly expands the potential for detailed investigation and monitoring diet not only in giant panda, but other species where separating certain taxa in the diet is difficult.

eDNA metabarcoding — a new approach to monitoring restoration

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Recent decades have seen a marked increase in both the scope and complexity of ecological restoration projects, partly in response to the increasing scale and severity of degradation to natural ecosystems. Individual restoration projects now operate over scales from hectares to hundreds of square kilometres and are increasingly underpinned by diverse and multidisciplinary science. These projects must be supported by measurable and realistic outcomes and standardised, accurate, and reliable approaches to their monitoring. However, studies indicate that monitoring is conducted ineffectually, or not at all, resulting in a poor return from restoration investments. Application of molecular tools has made important contributions to understanding factors influencing restoration success. Here, we outline how environmental DNA (eDNA) metabarcoding has the potential to revolutionize the practical contribution of genetics to monitoring in a restoration context. This talk outlines limitations of current approaches to monitoring biodiversity throughout ecological restoration, including their limited focus on the return of vegetation. In a restoration context, we are at the nascent stages in the application of eDNA surveys to establish baselines, monitor fauna during operational phases, and to track restoration chronosequences trajectories. We also briefly discuss current limitations (e.g., assay design and taxonomic reference databases) to a DNA-based approach to biodiversity assessment in restoration.

Comparison of parataxonomy and metabarcoding methods to assess the beetle biodiversity of Qinling Mountain

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Background: Metabarcoding is a powerful and fast tool for analysing biodiversity. However, the methodology is sensitive and is susceptible to false positives and contamination. We chose two sites from Qinling Mountain (Feng City and Zhouzhi City) to use a standard trapping method to obtain bulk samples of beetles and use them as an example to compare the results of parataxonomy and molecular methods. **Results:** Reference morphospecies were selected for assembly of mitogenomes using a shotgun sequencing protocol, and assembled mitogenomes were placed into a phylogenetic tree together with a large set of external mitogenomes. The total database comprised 1248 morphospecies. The remaining specimens were sorted into seven size classes, and each was further subdivided into small batches of either 50 or 25 specimens, prior to taking images of each batch, bulk DNA extraction, and PCR-based metabarcoding, prior to clustering into operational taxonomic units (OTUs). In total, well over 20 000 beetles in nearly 600 batches were included in this analysis. In a subsequent step, the OTUs were linked to the images, using a procedure that links partially identified specimens in the images to the sequence data based on the co-distributed OTUs in the batches. This approach provides a more secure OTU count than un-divided bulk samples, and provides a validation of OTU identities via the images. Preliminary analyses showed that Feng City had higher values of all three alpha diversity indexes (Shannon's, Simpson's, and Inverse Simpson's) than Zhouzhi City. **Significance:** Parataxonomy is widely used for species counts and assessment of species turnover, but it is wrought with uncertainty. Metabarcoding is potentially more accurate in separating similar-looking species and connecting morphological types across multiple samples. However, the analysis is very susceptible to problems caused by incorrect reads. A combination of parataxonomy and metabarcoding analysis gives more insight than using just one of the methods.

Barcoding and biosecurity: a molecular approach to evaluate the role played by the aquarium industry in the spread of invasive macrophytes in South Africa

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Background: South Africa has known exotic macrophyte invasion since the 1800s when *Eichhornia crassipes* (Mart.) Solms was first introduced and became naturalized in the KwaZulu-Natal province. An increase in the frequency and extent of invasion events currently place the already-fragile indigenous aquatic ecosystems, and the services they provide, under severe pressure. Attention has been directed towards the aquarium industry as a major pathway for the continued introduction of non-native freshwater plants to the country despite preventative regulations like the NEM:BA Act of 2004 and other national programmes in place. This comes as adequate enforcement is hampered by challenges to definitive species identification. Thus, the utility of a DNA barcoding approach to aid in addressing these issues is assessed. **Results:** After extensive core plant barcode (matK and rbcLa) reference datasets were compiled and tested, sequences for 133 out of 141 plants (94%) collected during a survey of 10 aquaria in and around the Johannesburg metropolitan area were generated. Around 90% of plants could be assigned to species, with 4% categorized as 1a (Invasive Species), 4% as 1b (Invasive Species Controlled by Programme), and 2% as prohibited under the NEM:BA Act. An additional 7% was found to be on the Global Invasive Species Database (GISD) but had no NEM:BA category. **Significance:** Results presented not only have several implications for current management programmes, but also provide the foundation for metabarcoding applications. Utilization of the LifeScanner system, a mixed-technology solution developed with the aim of making this technique accessible, will be discussed.

DNA barcoding, HPTLC fingerprinting, and phylogenetic analysis of an endangered Talipot Plam (*Corypha* sp.) from southern India

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Background: The monocarpic genus *Corypha* L. (Talipot Plam), which belongs to the family Arecaceae, is a fast-depleting palm that is much

valued in the aspects of ecological, cultural, and ethno-botanical uses. Taxonomic identification in *Corypha* is complex, due to the existence of morphotypes, overlapping morphological features, and rare availability of reproductive characters. Therefore, the number of species in this genus remains to be conclusively determined. DNA barcoding studies on *Corypha* are very much limited in India, which also lacks the information on the potential phytochemical resources. Our study reports the first DNA barcoding, HPTLC fingerprinting profiles, and phylogenetic analysis of an endangered species. **Results:** The results from HPTLC fingerprinting of immature seeds of ethyl-acetate extract confirmed the presence of pentacyclic triterpenoids (β-amyrin, oleanolic acid, lupeol, and ursolic acid) and phytosterols (β-sitosterol and sigmasitosterol). The HPTLC fingerprinting of immature seeds of methanol extract showed the presence of five major bands, and further, these chemical compounds will be confirmed by LC-MS. The phytochemicals were analyzed with a CAMAG HPTLC system equipped with LINOMAT 5 applicator, TLC scanner 3, and winCATS 1.3.4 software. Similarly, 10 accessions each representing *Corypha* sp. (currently identified as *Corypha utan* Lam.) collected from different parts of Tamil Nadu, India were analysed by DNA barcoding using five chloroplast DNA markers (rbcL, matK, trnH–psbA, rpoB, and rpoC) and one nuclear marker (ITS2). The intra- and interspecific divergence, species resolution, and Bayesian analysis of each DNA barcode will be presented in single and multi-loci approach. **Significance:** Our study reinforces that DNA barcoding could be utilized as a potential tool to resolve not only the species complex but also to improve the conservation strategies of endangered species. The phytochemicals identified for the first time in this study will enable understanding of naturally occurring genetic variations.

Origin and loss of species and genetic diversity: insights from barcode-based inference of parasite communities of northern European seals

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Background: Small and geographically isolated populations lose genetic variation through inbreeding and genetic drift. Low genetic diversity may, in turn, leave endangered animals vulnerable to attacks by fast-evolving parasites and pathogens. The situation is, however, complicated by the fact that especially specialist parasites will be bottlenecked in parallel with their hosts, and because the parasite communities of rare hosts may become simplified due to random extinctions. We used COI barcoding to examine community structure and genetic variation in cestodes and acanthocephalan intestinal worms in different-sized seal populations with very disparate levels of genetic diversity: Arctic and Baltic ringed seals, Baltic grey seals, and Ladoga and Saimaa ringed seals. **Results:** In the case of cestodes, we found a different species to be present in the Baltic Sea and in the landlocked Saimaa and Ladoga populations, evidently due to the availability of different intermediate fish host species in marine and freshwater environments. The situation was more complex in the case of the specialist acanthocephalans—based on the barcode sequence data, three to four species of *Corynosoma* can be identified in each marine seal species or subspecies, while the landlocked and relatively small seal populations of Ladoga and Saimaa harbor two and one species, respectively. **Significance:** Our results suggest a direct link between long-term host population size and parasite diversity, and that extinctions of parasites may mitigate the adverse effects of small population size and low genetic diversity in endangered animals.

Evolutionary relationships of threadfin breams (*Nemipterus* spp., Nemipteridae) from the Egyptian Red Sea coast and eastern Mediterranean Sea

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Background: Threadfin breams (*Nemipterus* spp.) are brightly colored demersal fishes that are endemic to the Indo-West Pacific region as well as the Red Sea. Their diversity, cases of misidentification, and recent descriptions of new species motivated this study. The Suez Canal, which connects the Red Sea with the Mediterranean Sea, further adds uniqueness to the study of these fish species. For instance, *Nemipterus randalli* is known to have migrated from the former to latter sea through the Suez Canal (Lessepsian migration). In this work, partial sequences of the cytochrome c oxidase subunit I (COI) gene were used to study the evolutionary relationships of four *Nemipterus* spp. and to compare the genetic diversity of populations of *N. randalli* from the eastern Mediterranean with a population off the Red Sea coast near Hurghada, Egypt. **Results:** COI sequence divergence did not exceed 3.5% in other *Nemipterus* spp., but it was 7.6% in *Nemipterus bipunctatus*. A Maximum Likelihood tree resolved *Nemipterus* species into four clades representing the four species studied. Genetic diversity analysis of *N. randalli* revealed six haplotypes in the Mediterranean Sea, five haplotypes in the Red Sea, and one shared haplotype between the two seas. **Significance:** The COI divergence of 7.6% in *N. bipunctatus* is more than twice the threshold (3.5%) for teleost fishes. Therefore, this fish species qualifies to be flagged as having a cryptic population in the Egyptian Red Sea coast off Hurghada and warrants a review of its taxonomic status. The shared haplotype between the Mediterranean and Red Sea populations indicates recent Lessepsian migration of *N. randalli*. Indeed, six *N. randalli* haplotypes detected in Mediterranean populations imply multiple invasion events of genetically diverse populations from the Red to Mediterranean Sea and advance the understanding of patterns of Lessepsian migration.

DNA barcoding of Portuguese Neuroptera

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Background: Neuroptera is an order of insects comprising about 5500 species worldwide, 300 of which are present in Europe, and includes the lacewings, mantidflies, antlions, and their relatives. Larval and adult forms of this group are important predators of agricultural pests, such as aphids, mites, and other insects. Albeit their relative ubiquity and notable importance, information on the group is still lacking, most considerably regarding taxonomic, genetic, and distributional data of several species, even in Europe. As an example, out of the 5500 extant species, less than 7% show available DNA barcodes in BOLD. In the Iberian Peninsula, the situation is also dire, as of the over 120 species known, only about a third have DNA barcode sequences in the database, but only 33 specimens originate from the region. This project focuses on Neuroptera from Portugal and is conducted within the framework of the InBIO Barcoding Initiative (IBI), a DNA barcoding initiative developed in CIBIO/InBIO and aiming to DNA barcode all terrestrial invertebrate groups of Portugal. We carried out fieldwork to collect specimens of species currently unavailable and that have never been barcoded. **Results:** Within the InBIO Barcoding Initiative, we have DNA barcoded more than 50 species of seven families of neuropterans. Genomic DNA was extracted, and the barcoding mitochondrial COI gene fragment (658 bp) was amplified. DNA barcodes were sequenced using high-throughput sequencing (Illumina). Most species could be easily distinguished using the targeted sequence, but some cases of low divergence between species were also

detected. Cryptic diversity was found in some cases, especially between specimens from Iberia and central Europe. **Significance:** The DNA barcoding of Portuguese Neuroptera is directly assisting the application of DNA metabarcoding techniques towards a better understanding of food web complexity in Mediterranean ecosystems, and the identification of trophic relationships relevant for pest management.

DNA barcoding and genomics tools to unravel the Amazonian biodiversity

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Industrial activities in the Brazilian Amazon are highly regulated by governmental agencies. However, best conservation practices are hampered by the lack of knowledge about megadiverse areas. This is the case for mining operations in the eastern Amazonian Carajás region, a mosaic of national parks, indigenous peoples conservation areas, and nature reserves. To provide solid scientific data that contribute to the implementation of best conservation practices, Vale Institute of Technology (ITV) is developing reference DNA barcodes for the flora, cave invertebrates, and bats of the region, and establishing deeper genomic references for species that are endangered or with complex identification. For this purpose, nuclear and chloroplast or mitochondrial markers, low coverage to complete genomic sequencing, or RADSeq are used. For several endemic plants, deep sequencing approaches used for the identification of markers under selective pressure has been conducted. These methods also contributed to understanding how populations are structured and how gene flow between populations is affected by natural factors and industrial operations. Models of environmental distribution and how it may be affected by climate change were determined for several taxonomic groups, including plants and bats. Further, we are establishing eDNA methods, metagenomics, and metaproteomics data for environmental monitoring of different altitude field phytophysiognomies, areas under rehabilitation processes, and caves. Together, these data constitute the most profound molecular representation of any environment in Brazil. All of the data are being provided to the public, and their use will be critical to the improvement of the conservation status of such a unique collection of species.

DNA barcoding of freshwater stream fishes of the Belém area of endemism

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Background: The freshwater fish fauna for the Belém area of endemism should represent one of the better-known regional fish faunas of the Amazon basin due to its proximity to a major urban centre which contains the second-oldest museum in Brazil with biological collections. However, cryptic species have been suggested in various basins of the region in studies targeted on specific genera. As such, large-scale barcoding was performed for all stream fishes collected in the region by our research group between 2008 and 2017 with an aim to cover at least five individual samples from each morphospecies identified in each drainage basin. Standard barcode of life practices were applied to over 2000 samples for the COI-5P target fragment. **Results:** Various morphospecies presented significant intraspecific divergences inconsistent with delimitation as single species. These included *Apistogramma* cf. *caetei*, *Crenicichla* aff. *saxatilis*, *Hyphessobrycon copelandi*, *Hypopygus lepturus*, *Iguanodectes spilurus*, *Mastiglanis asopos*, *Moenkhausia oligolepis*, *Nannostomus trifasciatus*, and *Otocinclus hoppei*. **Significance:** The presence of various cryptic and overlooked species in the Belém area of endemism indicates that even for relatively well-sampled regional fauna in the Amazon, the inclusion of molecular identification methods can help better map out biodiversity. The data produced will also serve as a reference dataset for ongoing work by our group testing the efficiency of metabarcoding methods in Amazonian streams, as well as providing a useful reference for standard molecular identification that may be used to track potential fraud and stock sources in the ornamental fish trade.

DNA barcodes for nature's pest controllers: the social wasps

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Background: Social wasps perform important ecological services of pollination, pest control, natural reservoir of brewers' yeast, and bioindicators of environmental quality. They are understudied relative to other insects, especially in Africa and Latin America. It is also in these areas of the world that wasps' services as nature's pest controllers offer greatest promise for integrated pest management programmes. There are about 40 Polistine wasps in West Africa, and only two of these have DNA barcodes available. DNA barcoding is a fast and relatively cheap way for species identification. Universal barcoding primers exist, but often coamplify contaminants such as numts or *Wolbachia* when present; hence there is a need to develop wasp-specific DNA barcoding primers. Here, we develop wasp-specific barcoding primers for Polistines and test their utility in species from Africa, Asia, Europe, and South America. **Results:** Sixty-four (64) species of social wasps were barcoded, including 40 Polistines from Africa and 24 Polistines, Stenogastrines, and Vespines from Asia, Europe, and South America. The universal barcoding primers LCO1490/HCO1928 worked well for all the species except in the genus *Ropalidia*, where it coamplified *Wolbachia* present. The Polistine wasp primers successfully amplified all Polistines from the four continents including 10 species in the genera *Belonogaster*, *Polistes*, and *Ropalidia* from Nigeria. The Polistine wasp primers captured 616, 568, and 562 bp of the barcode region in the Polistine wasps without coamplifying the *Wolbachia* present in the genus *Ropalidia*. **Significance:** We provide first reference barcodes for Polistine wasps. We emphasize the value of DNA barcoding for social wasps, through the discovery of four new species, and discuss how these data pave the way for developing the biological tools and resources required for a better understanding, appreciation, and capitalization of the services offered by this much-understudied group of insects.

One gene to rule them all: 18S mini-barcode distinguishes marine macrophyte lineages

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Background: There is barcoding universality in the animal kingdom, with the mitochondrial region COI being standard among many taxa. However, there is no barcoding universality for photosynthetic eukaryotes, and lineages beyond angiosperms are not well studied. The lack of DNA resources to accurately identify photosynthetic eukaryotes such as marine macrophytes limited the use of DNA metabarcoding on marine environmental analyses. Here, we tested amplification and taxonomical differentiation of 127 marine macrophytes (Embryophyta, Rhodophyta, Phaeophyta, and Chlorophyta) using the recommended plant barcodes rbcL, matK, and trnL, plus the genes ITS2, COI, and 18S. **Results:** Barcode genes rbcL, matK, and 18S

amplified all lineages of marine macrophyte, while barcodes ITS2, trnL, and COI were restricted to a single macrophyte lineage. We report the performance of these barcodes to distinguish among lineages of photosynthetic eukaryotes and for correctly assigning the tested species to their reference sequences. Taxonomical assignment was performed using a phylogeny-based approach. **Significance:** Considering the restriction of available reference databases, and considering the need to identify widely separated lineages such as macroalgae from marine angiosperms, we provide recommendations on what mini-barcode to be used on degraded DNA from environmental samples to identify and trace marine primary producers.

ssPRIMER — an open-source, GUI-based software tool for the design of species-specific primers and Taqman probes with implications for eDNA research

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Background: Quantitative PCR (qPCR) is a technique that has been shown to have great promise in the field of environmental biomonitoring, more specifically in environmental DNA (eDNA) detection. Here, we present ssPRIMER (or species-specific PRIMER), an open-source, web-based software tool that can be used to design species-specific primer sets and Taqman probes for qPCR assays. A multiple sequence alignment can be imported by a user, and the tool will then guide the user through the process of designing and evaluating species-specific primer and probe sets with a user-friendly interface. The tool is designed to create primer and probe sets that maximize amplification efficiency for the target species (sensitivity) but minimize amplification efficiency for nontarget species (specificity). **Progress:** An early prototype of the tool is functional for designing primer sets, while laboratory and field testing of primer sets designed using the tool is ongoing. A preliminary version of the tool is openly accessible on Github at https://github.com/m-orton/ssPRIMER, and the tool will soon be freely accessible online using the interactive web application Shiny. **Significance:** Overall, ssPRIMER comes with several advantages over pre-existing tools, including being freely available. Additionally, it uses the web-based platform Shiny, thus avoiding operating system-specific compatibility issues. ssPRIMER also provides useful evaluation options for the designed primer sets, including annealing temperature curve analysis and primer and probe binding visualization. Lastly, it offers simplicity in the user interface design and provides helpful instructions throughout the design, evaluation, and export process. This tool is designed to benefit the users of eDNA technology, including field biologists, ecologists, conservation researchers, and environmental consultants and could contribute to environmental biomonitoring using molecular methods.

Trophic ecology of African large herbivores: a continent-wide comparison from DNA metabarcoding

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Background: Large mammalian herbivore (LMH) communities in Africa are thought to be structured by resource competition and niche separation. Yet, the dietary niche has multiple dimensions: LMH populations may separate in time and space across habitats, in the utilization of plant functional groups, and in the selection of tissues. Interspecific separation at each of these levels has been invoked to explain LMH community structure, but the evidence is murkiest with respect to partitioning at the level of plant species. This dimension of the niche can be important to limit interspecific competition and promote species coexistence. Historically, the difficulty of identifying food items has been a major obstacle to resolving the taxonomic dimension of trophic niches. Molecular methods for diet analysis now enable trophic interactions to be characterized with high coverage and taxonomic resolution. In this study, we used faecal DNA metabarcoding to reconstruct plant–LMH interaction networks in 10 different protected areas throughout southeastern Africa, and investigate general patterns of LMH trophic ecology. **Results:** We collected high-resolution diet data from ~25 large LMH in seven different countries. Despite marked differences in herbivore species assemblages and resource composition/availability, interspecific segregation in plant–species utilization was observed in almost all communities, particularly in relatively stable communities. The partitioning was generally less pronounced among grazers than nongrazers, and can be highly variable between seasons. We further explored whether certain community properties (e.g., the overdominance of some species) and local parameters tend to be associated with stronger or weaker partitioning. **Significance:** Several generalities regarding the trophic ecology of large herbivores emerged from this continent-wide comparison study. We provided evidence that resource partitioning among large herbivores at the level of plant taxa is a common feature in most African savannas. These results provide new insights on assemblage rules governing LMH community assembly, and mechanisms promoting coexistence.

Developing new eDNA-based ecological assessment tools for the management of land-based contaminants in the coastal environment

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Background: Declining water quality is a major threat to coastal biodiversity. Contaminants are numerous, and their cumulative impacts on communities are poorly understood. Besides, traditional monitoring approaches present multiple constraints that limit observations to a small number of taxa. To implement effective management actions, there is a need to improve the description of coastal communities and disentangle the combined effects of contaminants on their structure/composition. Here, we propose to combine environmental DNA (eDNA) data—to describe coastal communities in a comprehensive and standardized way—with field measures of contaminants into cutting-edge statistical models allowing to disentangle effects of each contaminant on communities, and identify key stressors. This study aims to demonstrate the feasibility and practical potential of this approach. **Results:** Three estuaries, receiving different levels/types of runoffs, have been sampled (10 sites per estuary) in northern Queensland (Australia). At each site, we collected six water and sediment samples for eDNA analyses as well as a suite of contaminant measures (metals, nutrients, pesticides, etc.). Multiple taxonomic groups of interest were targeted to characterize community composition, and a range of biodiversity metrics was derived. These data are then combined into Bayesian Network Relative Risk Models.

These models can integrate multiple stressors, weight their relative importance on specified endpoints (here, biodiversity/community metrics), and rank their importance in each location. This information can then be used to make predictive models and explore effects of different managerial scenarios. **Significance:** The notion of studying a single stressor on a small number of taxa is now out-dated; more integrated approaches are needed. This research aims to provide a proof-of-concept for the integration of eDNA biodiversity data into ecological risk assessment models at a scale relevant for managers. This approach can provide comprehensive information on biological communities as well as key information for guiding mitigation actions and developing effective management strategies.

Horizontal transfer of chloroplast genome flux causes confusion for plant DNA barcoding

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Background: DNA barcoding technology is used to classify and authenticate herbal plants and also contributes to prevent economically motivated adulteration (EMA) of those products, which is vital for the stable growth of bio-industry. However, underestimating the genomic complexity mediated by horizontal plastid genome transfer can cause undiscovered shortcomings in DNA barcoding and molecular taxonomy. **Results:** We assembled plastid and mitochondrial genomes of *Cynanchum wilfordii*, an herb used as a functional food in the treatment of menopausal disorders, and *C. auriculatum*, which is found in EMA of *C. wilfordii*. In both species, the mitochondrial genome contained sequences related to ~35% of the plastid genome, termed mitochondrial sequences of plastid origin (MTPTs). We identified dynamic and lineage-specific horizontal plastid–mitochondrial genome transfer of up to 75 kb that contributed to diversifying mitochondrial genome structure complexity across 81 plant species. Additionally, co-amplification of MTPTs caused a DNA barcoding paradox in which herbal products could be mis-authenticated or mis-positioned taxonomically. **Significance:** Our results demonstrate frequent and lineage-unique MTPT distribution and show that it is conserved due to slow mutation rates in plant mitochondrial genomes. Co-amplification of MTPTs and intraspecies diversity creates a DNA barcoding paradox or taxonomical mis-positioning by the very tool for authentication. We suggest guidelines for DNA barcoding in EMA regulation.

DNA barcoding of Iberian Trichoptera: documenting biodiversity for freshwater biomonitoring in a Mediterranean hotspot

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Background: Trichoptera is a moderately diverse order of aquatic insects, with aquatic larvae and small moth-like adults. This group is closely related to the order Lepidoptera, and comprises over 14 500 described species. Trichoptera larvae are found in a wide range of freshwater habitats and show differential sensitivity to pollution, and thus their diversity and abundance are widely used in biological freshwater monitoring. However, these monitoring studies rely on larval morphological identification, which is much more difficult than adult determination and in fact impossible in the many species whose larvae have not yet even been described. The applicability of DNA metabarcoding for molecular identification of taxa in biodiversity monitoring is expected to be high, but dependent upon the availability of DNA reference collections. In this context, and within the framework of the InBIO Barcoding Initiative (IBI), we are developing a DNA barcoding database focusing on Iberian Trichoptera. **Results:** We have collected more than 700 specimens covering 22 families of Trichoptera, of which over 500 were already barcoded. Genomic DNA was extracted, and the 5′-region of the mitochondrial COI gene (658 bp) was amplified in two overlapping fragments. DNA barcodes were generated using high-throughput sequencing techniques (Illumina). From the over 150 Iberian species barcoded, most could be easily distinguished using the targeted DNA fragment, although in some cases low divergence was detected between species of the same genus (e.g., *Ceraclea*). Also, cryptic diversity was observed in some genera (e.g., *Rhyacophila*). **Significance:** The DNA reference database of Iberian Trichoptera being generated is directly supporting the development of freshwater biomonitoring methods based on DNA metabarcoding. With over 60% of Portuguese species and 40% of Spanish taxa already barcoded, and the current effort to increase its coverage, the IBI database of Trichoptera is expected to become a fundamental tool in freshwater biomonitoring in the Iberian Peninsula.

Artificial reef monitoring structures (ARMS) providing insights on marine biodiversity and community structure

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This project aims to set up a network of artificial reef monitoring structures (ARMS) in proximity to marine research stations in order to monitor status and changes in hard-substrate communities in coastal environments. The scientific purpose of the project is to provide a potential early warning system for marine biological invasions by identifying newly introduced non-indigenous species (NIS) and track the migration patterns of already-known NIS in European continental waters. Stations were chosen in different European regional seas, as defined by the Marine Strategy Framework Directive (MSFD), ranging from the Mediterranean to the Baltic Sea. The ARMS were deployed during the summer season and autumn of 2018 and retrieved after 2–4 months, according to the standards and protocols established by the Smithsonian Institution. The plates from the ARMS were disassembled and photographed, and samples of both the motile and sessile communities were collected for molecular analysis. Additionally, physico-chemical parameters were measured at the time of retrieval. DNA extraction and PCR amplification followed, targeting different molecular markers such as the 18S rRNA (for identification of metazoa at the genus level), the COI (for identification of metazoa at the species level), and the ITS (for identification of fungi) genes. High-throughput sequencing was chosen for the subsequent processing of the amplicons. Results will shed light on the investigation of marine biodiversity patterns across the European coastal waters. Furthermore, the results will provide crucial information on the importance of ARMS as a tool for biodiversity assessment and as an early warning system for the movement and establishment of NIS. This work was conducted in the framework of the ASSEMBLE+ project and in particular in the Join Research Activity of the Genomics Observatories.

Expanding the range of bioindicator taxa by assigning indicator values to selected metabarcodes

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The increasing use of metabarcoding for environmental impact assessment contributes to the extremely rapid growth of data available from environments subjected to anthropogenic pressures. These data contain metabarcodes of many potentially promising bioindicator taxa that have been overlooked until now due to their inconspicuous character and lack of indicator values assigned to them. Here, we explore these metabarcoding databases as a source of new bioindicator taxa of organic enrichment in marine coastal environments. Our study focuses on marine benthic meiofauna from the coast of Norway. We identify several meiofaunal species belonging to foraminifera, nematodes, and flatworms, whose distribution is strongly affected by the organic enrichment gradient. We assign indicator values to these taxa based on their occurrence in metabarcoding datasets with associated organic enrichment. These values correspond to the ecological categories used in most common benthic indices (AMBI, ITI, NSI). The results of this study contribute to expanding the reference database of these indices, considerably increasing the number of bioindicator taxa that could be used in metabarcoding surveys of marine environment.

DNA barcoding of the Israeli marine biota

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Background: In an era of fast global changes, the study of ecosystem functions and ecosystem services is in need of improved precision. We also need improved data accuracy for ecological and oceanographic models to predict future changes. Anthropogenic activity and climate change are already affecting the biodiversity in vulnerable marine ecosystems worldwide, including the shallow and deep sea Israeli Mediterranean and Red Sea habitats. To enable monitoring of these changes, the Israel Oceanographic & Limnological Research Institute (IOLR) initiated in 2013 long-term DNA barcoding campaigns for the marine biota in the Levantine basin of the eastern Mediterranean Sea, and for the coral reef biodiversity in the Red Sea. The Levant area is unique in being a hot-spot of tropical invasive marine species, entering to the Mediterranean Sea through the Suez Canal. The coral reefs in the Israeli Red Sea are of extremely high ecological and economical importance for the country. During the past six years, we carried out seasonal sampling of both the intertidal and hard-bottom shallow waters, together with deep sea surveys within the Israeli Exclusive Economic Zone (EEZ). **Results:** In total, an estimated 760 marine organisms were collected and catalogued in IOLR databases. Of these organisms, approximately 720 have been molecularly barcoded using the mitochondrial cytochrome c oxidase subunit I gene (COI). Among these are 478 fish, 280 invertebrates, and 3 algae. The vast majority of planktonic fish species are barcoded. Sequences and metadata are uploaded to the Barcode of Life Data Systems (BOLD). **Significance:** The data are used as a key tool for the National Monitoring Program, and by local authorities for identifying crucial gaps in knowledge. As such, they aid in management decisions for sustainable and proper use of resources in Israeli marine environments.

Holarctic or introduced? DNA barcodes provide insights on biogeography of beetles

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Background: Among the 8300 species of Coleoptera known from Canada and Alaska, most are endemic to North America. However, 640 are thought to be introduced, 401 are considered Holarctic, and the status of another 22 species is uncertain. Most of the introduced species are known or assumed to originate from the Palaearctic region. The extensive DNA-barcode libraries for European and Canadian Coleoptera provide an opportunity to explore the phylogeography of the beetle species shared by these continents to determine the status of the species of uncertain origin in North America, and to potentially pinpoint the geographic origin of adventive species. **Results:** The combined dataset for European and North American beetles includes 541 named species and 559 BINs with representatives from both continents. The sample size and geographic coverage of sampling are still limited for many of these species, especially in the Palaearctic. Most species considered Holarctic show separate barcode clusters between continents, with a varied levels of sequence divergence. Evidence of dispersal in both directions is evident in at least one Holarctic species. North American specimens of some species thought to be introduced from Europe are deeply divergent from all European specimens, suggesting they are native to North America or possibly to Asia. The status of five of the 22 species of previously uncertain origin in North America can be resolved based on the current dataset. **Significance:** Knowing the status of a species (native vs. adventive) in a given region is relevant for conservation efforts and for monitoring potential invasive pests. As the global DNA barcode registry for species continues to grow and geographical coverage expands, opportunities for phylogeographic studies will be created on an unprecedented scale.

An initial comparison of morphological and molecular approaches to water quality and biodiversity assessment in southern Africa

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Background: Forests are known for their megadiversity, containing up to 80% of all terrestrial species. South African forests cover only 0.08% of the land area, yet account for 7% of known vascular plant diversity. They are split into >16 000 fragments across the country, with less than 30% protected and the remaining fragments under extreme anthropogenic pressure. These highly fragmented and threatened forests need quick and efficient methods of quantifying and monitoring spatial and temporal biodiversity dynamics, especially as traditional methods of biodiversity assessment are relatively slow and expensive. The SAFFMAP project aims to use DNA-based identification through metabarcoding to enable the rapid assessment of biodiversity from mixed, bulk samples of terrestrial and aquatic invertebrates, even without taxonomic identification. The project focuses on the community of species rather than the individual as mass-trapped invertebrate specimens are subjected to genetic analysis without pre-selection. **Results:** We present the results from the first proof-of-concept study in South Africa, initially focused on aquatic macroinvertebrates from three rivers and two forests in the eastern Cape. We compare methods of traditional morphology-based water quality assessment (SASS) against metabarcoding of environmental DNA (eDNA), picked samples, and unpicked bulk samples. Our results show that unpicked bulk samples recovered the highest number of molecular operational taxonomic units (MOTUs), which included the MOTUs found in picked samples and more, while eDNA had little MOTU overlap with the other methods. **Significance:** Unpicked bulk samples show promising results for rapid biodiversity assessment, even with a poor reference library, and recovered more MOTUs than other, more time-consuming methods (picked and morphological).

HACSim: iterative extrapolation of haplotype accumulation curves for assessment of intraspecific COI DNA barcode sampling completeness

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Background: Assessing levels of standing genetic variation within species requires good estimates of sample sizes for the purpose of accurate specimen identification using molecular techniques such as DNA barcoding. Unfortunately, sample sizes of 5–10 specimens per species typically seen in DNA barcoding studies are often insufficient to adequately capture a wide range of within-species genetic diversity as a result of both species rarity and poorly resolved species boundaries. One tool that can be utilized to gauge the extent of sampling completeness for a species is haplotype accumulation curves, which depict the degree of asymptotic behaviour as a function of both the number of specimens sampled and the cumulative mean number of haplotypes accumulated. **Results:** This talk outlines a novel iterative extrapolation simulation algorithm of haplotype accumulation curves, called HACSim (Haplotype Accumulation Curve Simulator) that can be employed to calculate likely sample sizes needed to observe the full range of DNA barcode haplotype variation that exists for a species. Using equal (uniform) haplotype and unequal (nonuniform) haplotype frequency distributions, the idea of sampling sufficiency, the sample size at which sampling accuracy is maximized and above which no new sampling information is likely to be gained, can be gleaned to determine the value on the x-axis of accumulation curves where haplotype saturation occurs. **Significance:** HACSim can be employed in two primary ways to estimate specimen sample sizes: (1) to simulate haplotype sampling in hypothetical species, and (2) to simulate haplotype sampling in real species mined from the Barcode of Life Data Systems (BOLD). While it is found that our algorithm is globally convergent, runtime is heavily dependent on initial sample sizes (and skewness of the corresponding haplotype frequency distribution). HACSim is currently under development and will be released as an R package for global use by the DNA barcoding community shortly.

Investigating the mangrove productivity paradigm in relation to socio-economically important US fisheries

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Two thirds of the human population live in or near coastal areas, which has caused extensive damage to coastal ecosystems. In particular, mangrove ecosystems have undergone extensive damage due to anthropogenic pressure. Mangrove forests play an integral role for the sustenance of commercial fisheries and fuel such economies. Previous research has focussed mainly on the effects of abiotic fluctuations such as salinity and temperature on mangrove communities. However, relatively little is known about the nuanced interactions between mangrove ecosystems and associated organisms. This project aims to map out trophic interactions of heterogeneous subtropical mangrove ecosystems, identify sources of energy exchange, and predict the consequent impact on coastal fisheries. The aims of the project will be achieved firstly through metabarcoding of gut contents from key mangrove species using a combination of COI, 12S, and 18S markers. Along with gut samples, sediment and water samples will be collected to create a reference database, which will provide biodiversity information on organisms that occupy the surrounding waters. Carbon and nitrogen isotope ratios will be analysed to reveal the main carbon resource pool and determine trophic positioning. This research will be conducted in Estero Bay, adjacent to the Gulf of Mexico, in collaboration with Florida Gulf Coast University. Estero Bay is surrounded by mangrove forests that are fed by different rivers, offering an ideal juxtaposition of contrasting habitats. The results obtained will unravel vital information about how food webs are influenced by mangrove ecosystem heterogeneity and possible impact on fisheries recruitment.

Multilocus DNA metabarcoding of Pleistocene mammoth dung provides an integrated insight into changing Arctic vegetation and fungal–host interactions over time

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Although the Arctic ecosystem is currently low in biodiversity and productivity, this has not always been the case. During the Pleistocene, the vegetation was productive enough to support a diverse mammoth steppe megafauna. Ancient DNA metabarcoding of megafaunal fossilized dung has emerged as a vital tool to reconstruct Arctic vegetation history. Most of the previous studies, however, focused on one plant-specific DNA marker region, most commonly the P6 loop of the trnL plastid region. This may not always give a complete picture of all the vegetation present in the studied samples. Furthermore, fungal diversity is overlooked even though this plays an important role in nutrient cycling of ecosystems. In this study, we use a multilocus DNA metabarcoding approach, targeting both nuclear ribosomal (ITS1, ITS2) and plastid (rbcL, trnL) DNA to identify plants, fungi, and their host relationships. We study several permafrost-preserved Pleistocene mammoth (*Mammuthus primigenius*) dung samples and compare these to material from Holocene herbivores. Dung of fossil and modern Caribou (*Rangifer tarandus caribou*) is included for methodological validation. This approach will allow us to test whether any of the marker regions may be more resistant to degradation over time than others. The P6 loop of trnL is expected to be the most resistant, but preliminary results show that several plant genera that are missed by trnL are amplified by nrITS or rbcL. The results of this study will provide the most complete picture of a now-extinct Arctic vegetation and a first glimpse of its fungal–host interactions.

Reference sequence databases: a limiting factor in DNA metabarcoding?

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Background: The increasing popularity of metabarcoding warrants a careful look at the underlying reference databases used to make high-throughput taxonomic assignments. The objectives of this study are to document trends and assess the future usability of COI sequence records for metabarcode identification. **Results:** The number of COI records deposited to the NCBI nucleotide database has increased by a geometric average of 51% per year, from 8137 records deposited in 2003 to a cumulative total of ~2.5 million by the end of 2017. About half of these records are fully identified to the species rank, 92% are at least 500 bp in length, 74% have a country annotation, and 51% have latitude-longitude annotations. We describe the current taxonomic coverage in BOLD and GenBank. We introduce a new version of the COI classifier that merges sequences from both databases as well as a web portal for easy implementation of the COI classifier. **Significance:** To ensure the future usability of COI records in GenBank we suggest

the following: (*i*) improving the geographic representation of COI records, (*ii*) improving the cross-referencing of COI records in the Barcode of Life Data System and GenBank to facilitate consolidation and incorporation into existing bioinformatic pipelines, (*iii*) adherence to the minimum information about a marker gene sequence guidelines, and (*iv*) integrating metabarcodes from environmental DNA (eDNA) and mixed-community studies with existing reference sequences. The growth of COI reference records over the past 15 years has been substantial and is likely to be a resource across many fields for years to come.

Metabarcoding-based assessment of airborne pollen assemblages

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Background: Airborne pollen is a common trigger for both allergic rhinitis (hay fever) and asthma, which globally affect 400 million and 300 million people, respectively. Accurate pollen forecasts are important in managing these conditions, enabling sufferers to minimise their exposure. However, current UK pollen forecasts are based on counting individual grains under a light microscope, which is time consuming and requires highly trained personnel. Moreover, it is often not possible to morphologically identify pollen grains to the species or genus level, and counts may not be consistent between different collectors. Here, we assess the use of DNA metabarcoding as an alternative to light microscopy that avoids these drawbacks. **Results:** In this study, pollen was collected at up to 12 sites across the UK over multiple years and analysed using both metabarcoding and light microscopy. Airborne pollen was dominated by a few groups of wind-pollinated species, but in some instances high levels of pollen from insect-pollinated plants were also present. Pollen assemblages varied considerably across the season, but also differed between sampling sites. We demonstrate that metabarcoding and light microscopy were broadly in agreement about the time window over which pollen of a given family was present in the air. **Significance:** These results suggest the potential for high-throughput sequencing to be incorporated into current workflows for generating pollen forecasts, reducing costs and avoiding the limitations of microscopy-based pollen counts. By contributing to improved pollen forecasts and a better understanding of seasonal pollen dynamics, a better understanding can be developed of the impact of airborne pollen on human health.

The Criconematina Project: DNA barcoding of terrestrial nematodes

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DNA barcoding of terrestrial nematodes using COI has lagged in development compared with other groups of invertebrates. With fewer than 8000 nematode records with DNA sequences in BOLD, and an estimated 1–5 million species believed to represent total global diversity, the nematode barcoding gap is significant. To reduce that gap, we have developed a COI reference library for plant–parasitic nematodes in the suborder Criconematina. This suborder of root-feeding nematodes is global in distribution, abundant in tropical, deciduous, and conifer forests and remarkably diverse in native North American grasslands. To construct the reference library, individual nematodes were photographed, measured, and processed for PCR to generate a 721 bp barcode. The current library, representing 1700 specimens, has revealed insights into nematode biogeography and diversity. Criconematid nematodes have clear phylogeographic and biogeographic patterns that run counter to the prevailing narrative of widespread cosmopolitan distributions. There is little genetic evidence for long-distance dispersal of nematodes that are not associated with agricultural commerce. Endemicity, as recognized by local or regional haplotype groups, is common. Some haplotype groups display a close association with particular plant assemblages or individual plant species. Notably, the genus *Mesocriconema* exhibits significantly high levels of diversification and biome conservatism within North American grasslands. Another consistent theme in barcoding analyses of nematodes is the recognition of cryptic species. Many taxa originally defined on the basis of their morphology can be subdivided into morphologically similar, but genetically discrete, species. Consequently, DNA barcoding of nematodes will substantially increase the known diversity of this hyperdiverse phylum.

FreshBase: building a genome-ready reference collection of UK freshwater macroinvertebrates

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Background: DNA-based identification has the potential to revolutionise our understanding of biodiversity, but reliable names are only possible if specimens have been identified by experts, these vouchers are publicly available to reassess, and the resulting sequence databases have the relevant DNA sequence data. The FreshBase project aims to rectify this by collecting, identifying, preserving, and genome skimming representative specimens of all 4200 UK freshwater macroinvertebrate species, providing reliable names with multi-gene sequences and open-access DNA extracts that can be reused if/when marker choice changes in the future. **Results:** Through collaboration with multiple stakeholders, including government agencies and citizen scientists, the FreshBase project now includes a growing collection of vouchers in both ethanol and liquid nitrogen storage with associated open-access sequence data in major repositories. **Significance:** By building an open-access and marker-flexible repository for gold-standard vouchers, future DNA-based identification will be built on a solid foundation, enabling reliable interpretation.

Employing DNA barcode markers for identification and authentication of herbal samples of traded Indian medicinal plants

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Background: The National Medicinal Plant Board of India reports more than 7000 plants as medicinal, of which 960 are traded and with 178 having trade volume of more than 100 metric tonnes per annum. Impetuous collection of these from the wild has affected their natural populations adversely, and many of these have become rare. Consequently, instances of substitution/adulteration by look-alike substitutes have increased. DNA barcoding could be a potent tool for deciphering the botanical identities of herbal samples, invariably available in fragmented or powdered form. **Results:** A DNA barcode library was prepared by in silico analysis of the available sequences of four barcode markers, viz. ITS, ITS2, matK, and rbcL, in GenBank, NCBI of the 960 traded medicinal plant species, including 178 species of high trade volume. The sequences of one or more locus/loci of 438 species and all the four loci of 144 species were available. Individually, ITS provided the maximum species-specificity to 83% of the

438 species, and the three-locus combination of ITS+matK+rbcL was species-specific for 96.5% of the 144 species. As a result of this meta-analysis, DNA barcodes of 433 traded plants, including 115 highly traded plants became available. The barcode library developed was used for authenticating the botanical identities of 163 herbal samples, procured from different markets or online, supposedly belonging to 54 species, including 41 species of high trade volume by genetic distance and phylogenetic tree methods. Herbal samples (147) of 53 species could be tested, and 89 (60.5%) of the tested samples were authentic. **Significance:** Some of the herbal samples were substituted with their known substitutes or other unrelated medicinal plants, while a few by totally unrelated plant species, such as Besan (*Cicer arietinum*) in place of Vachhnag (*Aconitum ferox*) and an obnoxious weed, *Parthenium hysterophorus*, substituting for Pashanbheda (*Bergenia ligulata*).

Dietary assessment of groupers in Raja Ampat: insight from DNA metabarcoding

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Raja Ampat is well known as the most diverse site in the world for marine biodiversity, forming part of the Coral Triangle geographical region. Around 1508 fish species and 537 coral species have been found in Raja Ampat but are under threat from overfishing and other stressors. Reviews of key species in fisheries have been achieved in several places around the Coral Triangle. Food web analysis could facilitate understanding of ecology, and having an accurate technique for describing dietary components is essential. The advance of DNA metabarcoding has made this approach more powerful. The aim of this study is to identify grouper's diet via gut content analysis using DNA metabarcoding. Seventy-five Serranidae fish were collected from a fish landing in Raja Ampat for gut content analysis. PCR of the gut contents was performed using MiFish universal primers and invertebrate primers; this was followed by high-throughput sequencing on an Illumina MiSeq platform. We will present our findings, revealing new details of the dietary diversity and composition for groupers inhabiting this globally significant marine biodiversity hotspot.

High-resolution assessment of fish eDNA distribution in an Alpine river

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Background: Assessing the population density of fish species within rivers is important for the management and conservation of fish populations. Recently, environmental DNA (eDNA) has been evaluated as an important alternative and(or) complementary approach to traditional fish monitoring such as electrofishing or netting. Using eDNA to spatially characterize fish populations within a river demands to understand how eDNA distributes within it, especially at a small scale. However, this is yet poorly understood, as most studies focus on large-scale longitudinal distributions of eDNA (typically several km). **Results:** Here, we conducted a cage experiment in a fish-free river in Tyrol (Austria) during summer, autumn, and winter discharge situations. Four different fish species were caged for these four-day experiments, and water samples were taken daily from the cages up to 1.3 km downstream. A high-resolution sampling scheme was implemented, and water samples were taken at several distances from the cages, including multiple samples across the riverbed. eDNA of fish was detected and quantified using species-specific endpoint PCR followed by capillary electrophoresis (CE-PCR). We found that eDNA is distributed very heterogeneously close to its source due to limited water mixing. With increasing distance, the signal becomes more balanced at a lateral level. Moreover, the eDNA signal strength diminishes with increasing distance from its source, albeit this pattern varies depending on the discharge situation. **Significance:** The data obtained from this experiment provides the cornerstones for the interpretation of fish-eDNA signals obtained from Alpine rivers, as both the longitudinal and lateral distribution were examined during distinct discharge situations, hence enabling improved semi-quantitative assessments of fish populations based on eDNA.

DNA barcoding of *Glossogobius* spp. from seven lakes in the Philippines

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Background: *Glossogobius* is the most speciose genus of the family Gobiidae. Seven species of *Glossogobius* have been reported in Philippine freshwater lakes and rivers; the most important of which are *G. aureus*, *G. giuris*, and *G. celebius*, because these are widely abundant and are caught and sold for human consumption. **Results:** *Glossogobius* specimens were collected from seven lakes in the Philippines. A total of 56 specimens were DNA barcoded using the cytochrome c oxidase subunit I gene. Twenty-seven additional *Glossogobius* sequences were mined from GenBank. Based on morphological analysis and DNA barcoding, the majority (52) of the specimens were identified as *G. aureus*. Only four specimens collected from Taal Lake were identified as *G. celebius*. A neighbor-joining (NJ) tree using Kimura two-parameter (K2P) distances showed that all the specimens from Taal Lake, Naujan Lake, Lake Bato, Lake Buhi, and Paoay Lake clustered with *G. aureus* reference sequences with 99% bootstrap support. All the 10 specimens from Lake Mainit formed a single group distinct from the *G. aureus* cluster. The average K2P distance between the Lake Mainit group and *G. aureus* cluster is 3.8%. Two of the five specimens from Lake Lanao grouped with the *G. aureus* cluster, while the remaining three specimens formed a separate cluster. The average K2P distance between this separate cluster of Lake Lanao specimens and the *G. aureus* cluster is 3.6%. **Significance:** *Glossogobius aureus* was named and described by Akihito and Meguro only in 1975. This species is commonly mistaken for *G. giuris*. Based on the NJ tree, *G. giuris* specimens from India grouped separately from the *G. aureus* cluster. Akihito and Meguro included specimens from Laguna de Bay, Lake Lanao, and Taal Lake when they described *G. aureus*. This study revealed that *G. aureus* could be a species complex with at least two additional species from the Philippines.

Microsporidian infections in the *Gammarus roeselii* species complex (Amphipoda) over its geographic range: evidence for both host–parasite co-diversification and recent host-shifts

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Background: Microsporidia are obligate endoparasites. Both vertical and horizontal transmission routes are known. While the former may promote co-speciation and host-specificity, the latter may promote shifts between host species. Freshwater amphipods are hosts for many microsporidian species. However, no general pattern of host specificity and co-diversification is known. In southeastern Europe, *Gammarus roeselii* is comprised of 13 cryptic lineages of Miocene–Pleistocene age, but only one lineage has spread postglacially throughout northwestern Europe. Based on nearly 100 sampling sites covering its entire

range, we (*i*) explored the Microsporidia diversity in *G. roeselii* and their phylogenetic relationships, especially relative to parasites infecting other gammarids; and (*ii*) tested if host phylogeographic history might have impacted host-parasite associations (e.g., co-diversifications or recent host shifts from local fauna). We used a part of the small subunit rRNA gene to identify and determine the phylogenetic position of microsporidians. **Results:** Microsporidian diversity was high in *G. roeselii* with 24 detected haplogroups, clustered into 18 species-level taxa. Ten microsporidia species were rare, infecting few individual hosts in few populations. Most of them are phylogenetically related to parasites from other amphipods or crustaceans. Others were widespread genera with high prevalence: *Nosema*, *Cucumispora*, and *Dictyocoela*. Two contrasting host association patterns could be observed. First, two vertically transmitted species, *Nosema granulosis* and *Dictyocoela roeselum*, share the pattern of infecting *G. roeselii* over most of its range and are specific to this host. It suggests the co-diversification scenario. This pattern contrasted with that of *Dictyocoela muelleri*, the three species of *Cucumispora*, and the rare parasites, present only in the region recently colonised by the host. These patterns suggest recent acquisitions from local hosts. **Significance:** Microsporidia infecting *G. roeselii* revealed two scenarios of host–parasite associations: ancient associations with vertically transmitted parasites that probably co-diversified with their hosts, and host-shift from local host species, after the host's postglacial spread.

New avenues for data curation: hackathon on marine invertebrates

Adriana E. Radulovici,[1] Filipe O. Costa,[2,3] and the Hackathon Participants

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The use of DNA barcoding for the identification of unknown specimens relies on the existence of reliable databases in terms of taxonomic resolution and sequence quality. With technological advances and major international efforts in DNA barcoding, the amount of data generated is increasing exponentially, leading to a stringent need for data curation. Marine data present an extra layer of complexity for data curation since no genetic database has an easy option to filter marine data only. Here, we focus on the largest database of DNA barcodes (BOLD, Barcode of Life Data Systems) and present the results of the first data curation hackathon on marine invertebrates undertaken during a pre-conference workshop at the 8th International Barcode of Life Conference (Trondheim, Norway, 17–20 June 2019). Due to time constraints (one day) and in order to test the workflow for data curation, only major groups of marine invertebrates (polychaetes, molluscs, crustaceans) were targeted. The congruence of DNA barcodes with Linnean names was investigated, and the records in need of further scrutiny were annotated. More concerted effort for data curation should be considered in order to have a high-quality dataset for marine invertebrates, and the solutions discussed during the hackathon will be presented during this talk.

GTI-DNA-tech: building DNA barcoding capacity in developing countries through reinforcing local expertise

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National environmental protection authorities around the world are in need of rapid and accurate species identification methods to inform biodiversity conservation actions, such as conservation or management plans for priority species (e.g., endangered species, invasive alien species, pests, and pathogens). DNA barcoding has become common practice when identifying unknown specimens for research purposes; however, its global implementation as a standard regulatory tool for biodiversity conservation faces some challenges. One such challenge is the limited technical capacity in developing countries where a large portion of the global biodiversity is harboured, much of it still awaiting discovery. To address this challenge, the Secretariat of the Convention on Biological Diversity (CBD), in collaboration with the University of Guelph, launched GTI-DNA-tech—an initiative to facilitate hands-on training for experts in developing nations. During Phase I (2015–2016), specialized theoretical and practical courses in standard DNA barcoding workflows were delivered in Canada for 29 experts from 28 countries. In Phase II (2017–2018), 10 of these "trained-trainers" organized training courses in their home countries, using the standard training package developed by the GTI-DNA-tech partnership to disseminate the technology nationally. Overall, more than 100 institutions were involved in Phase II, with about 100 organizers and instructors training more than 150 experts. Two workshops were regional and brought participants from additional countries, resulting in 18 developing countries covered by GTI-DNA-tech in 2018. National environmental protection authorities were successfully engaged and committed to include DNA barcoding in respective national strategies for the implementation of the CBD. We present the outcomes of these 10 training courses and their sustainability plans to tackle the taxonomic impediment in biodiversity conservation for the concluding years of the United Nations Decade on Biodiversity (2011–2020) and beyond.

Assessing aerial tree pollen composition via metabarcoding analysis of environmental DNA

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Background: Airborne pollen is ranked as the world's most harmful aeroallergen, with pollen allergy presenting a significant socioeconomic burden. Contributing factors influencing allergenic reactions include the aeroallergen concentration and the taxonomic origin of pollen grains. In the UK, current pollen monitoring protocols aim to reduce this burden by monitoring and forecasting regional atmospheric pollen concentrations. However, due to morphological features being largely conserved across taxa, traditional palynological techniques are unable to discriminate between species of grass and tree pollen, and the ability to accurately characterise the taxonomic composition of airborne pollen remains an Achilles heel of pollen forecasting. **Results:** Using aerial environmental DNA (eDNA) sampling and DNA metabarcoding (with two complementary DNA barcode markers, rbcL and ITS2), we aim to explore the composition of airborne pollen across the tree allergy season, at two sites in Great Britain. We will compare traditional palynological techniques and targeted high-throughput sequencing to assess the accuracy of pollen identification via DNA metabarcoding and the ability to detect rare species. Furthermore, we will investigate the influences of bias involved in pollen DNA metabarcoding and test measures of association

between species' abundances to explore cross-utility of the different approaches for measuring pollen abundance. **Significance:** We anticipate that this research will increase our understanding of the ecology of airborne pollen in time and space and demonstrate the usefulness of DNA metabarcoding in pollen monitoring and aerobiology research.

Identification of five species of *Dalbergia* (rosewood) from Madagascar using molecular barcodes

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The identification of *Dalbergia* (Fabaceae) of Madagascar, including rosewoods (5 species) and palissander (more than 40 species), is problematic because the species are closely related to one another and have similar morphology. However, identifications of each species are essential for the country in order to control the illegal logging and international trade of these precious woods. Molecular tools are currently the solution to this problem. To date, the project to identify the *Dalbergia* from Madagascar is in progress. Leaf and wood samples were collected in the northern part of Madagascar and will be the subject of a molecular study to establish a solid DNA database for conclusive species identification. To perfect the results, the samples chosen are the most commercial species and have been almost identified at the morphological level. DNA extraction assays on *Dalbergia emirnensis* showed fairly high quality (unquantified but migrated) using the MATAB protocol (modified Carlson); and amplification with standard markers such as matK, rbcL, and ITS showed good migration under electrophoresis. These pre-results indicate good prospects for further work. However, there are three major challenges to be addressed: (1) finding usable regions for the five species of *Dalbergia* DNA barcoding using the standard markers matK, rbcL, and ITS; (2) finding other loci that can be used for the DNA barcoding of *Dalbergia* species from Madagascar; and (3) finding a protocol for extracting DNA from wood. The obtained results will be used (*i*) for the sustainable management of precious woods in Madagascar, (*ii*) as a reference file for the identification of all other *Dalbergia* species, and (*iii*) for enriching the Barcode of Life Data Systems (BOLD) database.

Intricacies involved in authentication of herbal industry samples by DNA barcoding

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Background: DNA-based identification methods have been universally accepted for plant authentication by various world pharmacopoeias. This is due to the effectiveness of the DNA barcodes in identification of unknown adulterants and contaminants in herbal products. Thus, the time is right for herbal industries to adopt DNA barcoding permanently into their QC chain alongside existing taxonomical and phytochemical profiling to authenticate their bulk materials. **Results:** We have tested around 400 commercial samples belonging to a range of plant species and families, covering 23 different types of tissue samples like leaves, root, bark, fruit, etc. The samples were of fresh, dried, extracted, and powdered form, for which a standard experimental DNA barcoding protocol was employed. We found that identifications were as expected for most samples, but that a few samples were difficult to classify. The intricacies were overcome by interacting closely with industry to arrive at species-level confirmation. In one case, we observed 100% hit score with one universal DNA barcode marker, while the same sample matched with a different species with a different DNA barcode marker. For example, similarities between *Amaranthus hybridus* and *A. tricolor* was 99.3% sequence identity with ITS2, and the same showed 100% similarity when rbcL was used, although they are morphologically distinct. Secondly, *A. hybridus* is difficult to distinguish from the cultivated taxa, *A. hypochondriacus*, *A. cruentus*, and *A. caudatus*. **Significance:** From this study, it can be concluded that some intricacies observed by using DNA barcoding can be effectively overcome by the correct usage of synonyms for the species, and by considering interferences caused by microbial contamination, etc. Awareness of such complexities will help the commercial DNA barcoding of herbal products.

DNA barcoding of leafhopper vectors (Hemiptera: Cicadellidae) of phytoplasma on ornamental crops

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Background: DNA barcoding is increasingly used for quick and accurate species diagnosis when morphological similarities often lead to misidentification. In this regard, leafhoppers are the primary vectors of phytoplasma on various horticultural crops, including ornamentals like China aster, marigold, and chrysanthemum. Different species of leafhoppers are not easily identified due to their cryptic nature, requiring a specialist to dissect genitalia for species diagnosis. This taxonomic impediment is removed by DNA barcoding employing any developmental stages of the target species. **Results:** Leafhoppers were collected on marigold, chrysanthemum and China aster during different phenological stages of these crops during 2017 from Karnataka state, India, and preserved in dilute alcohol (70%) until further studies. Total genomic DNA was isolated from individual specimens and amplified using mtCOI (LCO/HCO) primers, sequenced, and analyzed using BioEdit. In the current study, unequivocal identification was made of the following leafhopper species in Karnataka viz. *Sogatella furcifera*, *Homalodisca insolita*, *Balclutha incise*, *Amrasca biguttula*, *Toya* sp., *Empoasca* sp., *Sogatella* sp., *Perkinsiella* sp., *Hishimonus* sp., *Tambocerus* sp., *Phaconeura* sp., *Curena* sp., *Psammotettix* sp., *Graphocophala* sp., *Balclutha abdominalis*, and *Japanagallia trifurcata*. **Significance:** Identification of the correct species of leafhopper is important from the point of view of field-level transmission of phytoplasma for studies on the epidemiology, vector–phytoplasma interactions, biological control, and finally integrated management of phytoplasma. Since both leafhopper vectors and phytoplasma can occur on a multitude of crops, it is important to understand the nature of association of the leafhopper vectors, phytoplasma, and the crops. This impediment is effectively resolved by DNA barcoding.

DNA barcoding in the early detection of the invasive alien species, the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) and its strain status in India

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Background: Invasive alien species (IAS) pose serious threats to global agriculture, mainly affecting the livelihood of small and marginal farmers. Quick and accurate species diagnosis is important to take effective mitigation measures on these farms. The fall armyworm, *Spodoptera frugiperda*, appeared as a pest on corn and sorghum in India in June 2018. The species was detected in Africa in 2016, and was not expected to occur in India so soon. This ignorance contributed to the big outbreak observed in India in 2018. The lack of effective measures was also partly due to the morphological similarities of *S. frugiperda* with other noctuids occurring in high numbers during previous years. **Results:** We employed DNA barcoding to identity *S. frugiperda* on different crops such as corn, rice, sugarcane, areca nut, and marigold from six states of India: Karnataka, Tamil Nadu, Andhra Pradesh, Telangana, Maharastra, and Madhya Pradesh. We also employed DNA barcodes to decipher the strain status of the species. A total of 22 barcode sequences was generated and submitted to NCBI. In addition, based on DNA barcodes, we have demonstrated the prevalence of the rice strain of *S. frugiperda* in India. **Significance:** Establishing the species identity of invasive pests like *S. frugiperda* is of

foremost importance, as it damages more than 350 host plants, principally corn and rice. In addition, identifying strains of *S. frugiperda* in India is important as the recognized rice and corn strains differ in crop preference, genomic details, response to pheromones, resistance to insecticides, and biological control.

Characterization of the complete chloroplast sequence of *Juniperus excelsa* subsp. *polycarpos* M. Bieb (Cupressaceae)

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Background: *Juniperus excelsa* subsp. *polycarpos* M. Bieb., known as the Persian juniper, is dominating in the northern mountain ranges of Balochistan and declared the second-largest living fossils of Juniper forest. The plant is important medicinally and ornamentally. However, there are few studies on the chloroplast genome of *Juniperus*. **Results:** We sequenced and analyzed the complete chloroplast genome of *J. excelsa* subsp. *polycarpos*. The chloroplast genome of *J. excelsa* subsp. *polycarpos* is a circular molecule of 127 384 bp in length with 130 single-copy genes and six duplicated genes (trnI-GAU, trnA-UGC, trnL-UAA, trnF-GAA, trnV-UAC, and trnQ-UUG). The genome contains 82 protein-coding genes, four ribosomal RNA genes, and 27 transfer RNA genes. In these genes, nine genes (rpl2, ycf2, trnA-TGC, trnE-TTC, rpoC, rpoB, ndhB, ndhA, and atpF) harbor a single intron, three genes (accD, rrn23s, and ycf3) harbor two introns, and one gene (ycf1) harbors three introns. Like other sequenced chloroplast genomes of conifers, this genome does not contain canonical inverted repeats (IRs), and the overall GC content of *J. excelsa* subsp. *polycarpos* chloroplast DNA is 35%. The phylogenetic analysis revealed that *J. excelsa* subsp. *polycarpos* is more closely related to *J. scopulorum* and *J. bermudiana*. **Significance:** Our findings may provide baseline data for future research related to *Juniperus*, particularly in the Ziarat ecosystem of Balochistan, Pakistan.

DNA barcodes for identification of rosewood, palisander, and ebony (*Dalbergia* and *Diospyros*) from Madagascar

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Madagascar has an important biodiversity that is threatened by extinction, such as precious wood species. In 2013, rosewood, palisander, and ebony are introduced in Appendix 2 of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) due to lack of data on their species management and the increase of illegal exports. In order to control illegal logging, movement, and international trade of these woods, identification tools are needed. Currently, Madagascar starts a program (2018–2022) in collaboration with the European Union for the inventory and identification of existing and exploited precious wood species. The first step is to collect samples of plant material: leaves, wood, bark, and DNA throughout the Island. At the University of Antananarivo, a plant molecular biology laboratory has been refurbished and equipped. Right now, more than 200 samples are collected from the north of the country. During 2019, many more samples will be collected in the eastern, western, and southern regions of the country. The second step is the analysis of all collected samples. These samples are studied for the morphological characters, and DNA samples will be sequenced using the following loci: ITS2, rbcL, matK, trnL, trnH–psbA, trnV–trnM1, trnV–trnM2, trnC–petN, and trnS–trnG. The correspondence between the results will allow the establishment of reliable identification methods for the sustainable management of precious wood species in Madagascar, including timber traceability and making nondetriment findings (NDFs).

mBRAVE: the multiplex barcode research and visualization environment

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Background: Widespread interest in the study of metabarcoding has resulted in data proliferation and the development of a multitude of powerful computational tools. Yet the consistent and reproducible interpretation of the data remains challenging. The integration of different data types, software tools, and analytical parameters pose a barrier to scaling research. Further, though the majority of the necessary tools for performing these analyses are already implemented, there is limited support for high-throughput analysis due to the requirement for heavy computational capacity. As a result of these complexities, many researchers lack the time, training, or infrastructure to work with larger datasets. **Results:** mBRAVE, the multiplex barcode research and visualization environment, is a cloud-based data storage and analytics platform with standardized pipelines and a sophisticated web interface for transforming raw HTS data into biological insights. mBRAVE integrates common analytical methods and links to BOLD for reference datasets, presenting users with the ability to analyze large volumes of data without requiring special technical training. mBRAVE is built on cloud architecture, which provides centralized and automated storage and compute capacity, thereby reducing the burden on individual researchers. **Significance:** The mBRAVE platform seeks to alleviate the main informatic challenges faced by the metabarcoding research community — the storage and consistent interpretation of HTS data. It is now available for researcher use at www.mbrave.net.

Can sedimentary ancient DNA (sedaDNA) be used for sea ice reconstructions?

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Background: Records beyond observational time scales are essential for documenting and understanding the natural variations in Arctic sea ice extent. However, the tools to document the evolution of sea ice conditions on historical to geological time scales are few and have limitations. It makes that sea ice, although a crucial component of the Arctic and global climate system, remains incompletely understood. **Results:** Here, we have explored using sedimentary ancient DNA, or sedaDNA, as a novel tool for past sea ice reconstructions. We used 18S metabarcoding and single-species quantitative DNA detection methods to document the sea ice conditions in a Greenland Sea marine sediment core. Metabarcoding has allowed identifying biodiversity changes back to ~100 000 years ago that we relate to changing sea ice conditions. Detailed bioinformatics further revealed several sea-ice-associated taxa, of which several were previously unknown from the traditional (micro)fossil record. The results from our genetic approaches corroborate sea ice reconstructions by traditional tools, like dinoflagellate cyst assemblages and the sea ice diatom biomarker IP_{25}. **Significance:** In summary, we show that sedaDNA has great potential for documenting past sea ice conditions beyond instrumental time scales and provides a new tool to better understand past sea ice evolution.

German Barcode of Life project: legacy and progeny

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and J. Wolfgang Wägele, on behalf of the GBOL Consortium

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The German Barcode of Life (GBOL) initiative is a consortium of natural history museums and research institutions, funded by the German Ministry of Education and Science (BMBF) and is approaching its final steps. During a first (2011–2015) and second phase (2016–2019), a network of professional and nonprofessional taxonomists was established to start with the construction of the DNA barcode reference library for the fauna, flora, and fungi of Germany. In this talk, we will give a brief overview of the activities during the last years. More than 25 000 species of animals, plants, and fungi were collected in over 300 000 samples. From more than 90% of the collected species and around 70% of all samples collected, a DNA barcode could be generated. The web portal (www.bolgermany.de) provides access to the data and can be used for reverse identification approaches. During the second phase, several projects aimed at applying barcoding and metabarcoding techniques for the purpose of, for example, monitoring ecosystems and biodiversity. Especially in the context of the dramatic insect decline reported from German nature conservation areas—a hotly debated topic in politics and media—the increased need for fast biodiversity inventories and monitoring is obvious and striking. Yet, concerning the estimated number of species in Germany, only about 25% of Hymenoptera and about 33% of Diptera are covered in the GBOL barcode library. These gaps in the reference library come up as operational taxonomic units (OTUs) in the analyses of Malaise trap studies and should be targeted in future initiatives we will present. Other initiatives export the know-how and the routines established in GBOL towards other countries and ecoregions: one example is the Georgian-German Biodiversity Center (GGBC).

Beyond the DNA reference library of the German Barcode of Life project: methods and applications in progress

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The main goal of the German Barcode of Life (GBOL) initiative, a consortium of natural history museums and research institutions funded by the German Ministry of Education and Science (BMBF), was the establishment of a DNA barcode reference library for the fauna, flora, and fungi of Germany. In the second phase starting from 2016, 11 projects with seven PhD students put the database to the test for different applications. Acting as exemplary pilot studies, they cover a wide range of topics. In the context of legal aspects, crucial organisms for forensics, food control, and beverage production are pointed out and included in the DNA reference. Barcoding helped and improved pest controls by the detection of phytopathogenic fungi (aiming further at developing a diagnostic microarray chip) or by uncovering egg-parasitoids in *Trichogramma* wasps. The analyses of pollen might improve the analysis of air monitoring and, with regard to pollination, reveal relationships of plants and insects. Metabarcoding is used for water quality assessments in the context of the Water Framework Directive (macrozoobenthos, diatoms) or for biomonitoring via soil and Malaise traps. The value of the reference collection is improved as the organisms needed for these research questions are added to the database. At the same time, these studies successfully show that the DNA reference library does not act as an end in itself. The use of this reference library for application-oriented questions improves the quality and the speed of the answers. For instance, for national control authorities, legislation, ecosystem inventories, monitoring for nature conservation and agriculture, and many more applications, the successful establishment and application of DNA barcoding methods have been achieved.

Dietary overlap in commercial fisheries food-webs in the southwestern Atlantic

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Background: Determining dietary overlap between commercial fisheries species is important for understanding possible effects of fishing pressure in any given region. However, traditional stomach content analyses often fail to identify dietary items to low taxonomic levels. Metagenomic analysis provides a reliable method for defining overlap using either taxonomic associations or, when lacking a reference sequence, molecular operational taxonomic unit (MOTU) assignment. Stomach samples were obtained from over 250 individuals during onboard sampling in early 2016 as part of a broader project on ecosystem-based management for southeastern Brazilian pelagic fisheries. These represent 28 species, including not only the targeted pelagic species but also many benthic and demersal bycatch species. Where possible, at least three stomach samples were analysed per species as a first pass to describing the trophic preferences of each species. Sequencing was performed via an amplicon approach using three primer sets (Chord-16S, Ceph-16S, and MiniBar-COI) to maximise taxonomic coverage, and run on the IonTorrent S5 platform. **Results:** The diversity of dietary items was relatively consistent between individuals of the same species despite moderately low sample numbers. We present the results in terms of patterns of similarity and dietary overlap between taxa as well as between functional trophic groups and taxonomic groups of the commercial fish sampled. **Significance:** We also note that the information on biological diversity generated can also be used for future analyses of regional diversity trends and the identification of potentially undescribed taxa in conjunction with traditional molecular identification approaches, such as DNA barcoding. Signals of the same terrestrial organisms were regularly identified, likely indicating both natural wind-blown sources and probable anthropogenic contaminants.

DNA barcoding of Odonata in Poland — first step for the national freshwater macroinvertebrate reference library

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Background: The broad adoption of DNA barcoding is accelerating the documentation of animal diversity. While DNA barcode coverage for odonates is well advanced in some regions, current records from Poland are scarce. The dragonfly and damselfly fauna of Poland is well known, consisting of 74 species (26 Zygoptera, 48 Anisoptera), some represented by a few records. Recent expansion of southern species has also been observed, caused by the climate changes, including increasing mean yearly air temperature as well as shorter and warmer winter periods. Thus, we selected Odonata as a prefatory taxon in the development of the "Freshwater Macroinvertebrate DNA Barcode Library of Poland" coordinated and curated by our department team. **Results:** In 2018, we visited several sampling sites throughout Poland to collect dragonflies and damselflies. Larvae were recorded in 19 localities, including big rivers (Vistula, Oder), oxbows, ponds, peatbogs, and dam lakes. We identified 23 species (9 Zygoptera and 14 Anisoptera), which represent one third of already-known species occurring in Poland and almost 20% of European species. We selected ~150 individuals for COI barcoding to cover as evenly as possible the spatial distribution of the collected species in Poland. **Significance:** The study initiates the construction of a barcode reference library for the freshwater macroinvertebrates of Poland, that will provides species-level identifications as well as insight into plausible cryptic

diversity within freshwater taxa. This initiative, in compliance with the activities of the EU COST Action "DNAqua-Net", will fill an important and wide gap in the European barcoding efforts.

Odonata of Malta — filling a DNA barcoding gap in the Mediterranean

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Background: The three Maltese Islands, Malta, Gozo, and Comino, situated in the middle of the Mediterranean Sea, are naturally poor in surface fresh waters, due to geological and climatic conditions. Moreover, the aquatic and wetland habitats are degraded by various anthropogenic activities, but man-made water bodies (e.g., artificial ponds, reservoirs, canals) somewhat compensate the loss of the natural habitats. The Odonata fauna of Malta is well known, consisting of 19 species, but many of them are migratory and only represented by a few adult specimens. **Results:** In 2018, we visited 80 sampling sites on all the three islands, to collect aquatic macroinvertebrates, among them dragonflies and damselflies. The sampling sites were classified into five groups: (1) larger standing waters, (2) small standing water with surface area less than 1 m^2 (e.g., rock pools, puddles), (3) small artificial habitats, (4) running waters, and (5) salt marshes. Odonata larvae were found at only 25 sites (31% of the visited sites) and consisted of about 150 specimens. The most important habitats were the larger standing waters (larvae were found in 57% of them) and the small standing waters (40%). Odonata larvae were unexpectedly rare in flowing waters (26%). The number of species/sites was low in each habitat type, varying between one and four. Nine species belonging to three families were collected (one Coenagrionidae, two Aeshnidae, six Libellulidae). All the species have now been subjected to DNA extraction, amplification, and sequencing of the COI barcoding marker in order to provide a reference barcode library and to screen for the presence of plausible cryptic taxa. **Significance:** This is the first effort to generate a DNA barcode reference library of odonates of Malta that provides species-level identifications. The barcodes will become part of the "Aquatic Macroinvertebrates DNA Barcode Library of Malta" that is being developed on the basis of our sampling.

Trophic interactions of European hake in the Adriatic Sea by using metabarcoding

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Background: European hake (*Merluccius merluccius*) is a demersal fish widely distributed in the Mediterranean, and it is one of the most economically important fish for this basin. It is a voracious predator of deep upper shelf slope communities currently characterised by growth overexploitation; the understanding of hake's diet might support an ecosystem-based fishery management. However, all current European hake diet studies depend on the morphological identification of prey remains in stomach contents, with consequent limitations. **Results:** In this study, we set up a metabarcoding approach based on cytochrome c oxidase subunit I (COI) PCR amplification and Miseq Illumina paired-end sequencing of *M. merluccius* stomach content remains and compared the results to classic morphological analyses to assess its efficiency and accuracy. A total of 95 stomach contents of *M. merluccius* sampled in the North-Central Adriatic Sea was analysed with both the metabarcoding and morphological approaches. Moreover, a positive mock sample allowed us to evaluate the efficiency of this metabarcoding method for species identification in a known DNA pool. Metabarcoding clearly outperformed the morphological method in the taxonomic identification of prey, describing more complex trophic relationships even when considering the morphological identification of 200 stomach contents. Statistical analysis of diet composition revealed a weak differentiation among the hake's size classes, confirming an opportunistic feeding behaviour. All the analyses performed showed the presence of a core of shared prey among the size classes and a cloud of size-specific prey. **Significance:** Our study highlights the exceptional potential of metabarcoding as an approach to provide unprecedented taxonomic resolution in the diet of *M. merluccius* and potentially of other marine predators, due to the broad-spectrum of detection of the primers used. A thorough description of these complex trophic relationships is fundamental for the implementation of an ecosystem approach to fisheries.

How unbee-lievable, but I'm not pollen your leg! Using metabarcoding to reveal the role of nonbee pollinators in strawberry crops

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Background: Mounting evidence suggests that pollinator diversity is important for optimal ecosystem service and function. While bees are frequently the most efficient pollinators, due to their pollen-dependent diet, they represent only a small fraction of the pollinator diversity. Nonbee pollinators have received little recognition for their role in commercial agricultural pollination despite representing 95% of flower visitor diversity. Many nonbee pollinators are more resilient to land-use intensification and climate change due to their nomadic life-history and tolerance of inclement weather. Our research characterises nonbee pollinator communities, their foraging preferences, and floral fidelity in strawberry crops. **Results:** We caught a total of 640 nonbee flower visitors, across three field sites, during three months of the flowering period (May–August) of day-neutral strawberries in southern Ontario. Diptera and Hymenoptera (primarily bee species) were the most abundant flower visitors; Coleoptera and Hemiptera were also collected from flowers. We will link specimen identifications, determined by barcoding, to the abundance and diversity of pollen found on their bodies, and related to local meteorological data. We will use metabarcoding of pollen to determine the network of plant–pollinator interactions associated with strawberry flower visitors. Comparing pollen abundance counts with total read abundance of metabarcoding, we will examine whether it is possible to use metabarcoding to determine pollen abundance. **Significance:** We have advanced metabarcoding tools for pollen identification during this project that will be useful for future in-depth biodiversity and ecosystem service assessments. Our species-level identification of strawberry-flower-visiting insects and associated pollen samples will be made publicly available on BOLD, providing an invaluable tool for future pollinator assessments. Characterising the identity and diversity of nonbee pollinators provides a baseline for future pollinator assessments, allowing them to be included in passive sampling protocols.

DNA barcoding for the identification of non-indigenous marine species in Tunisia (southern Mediterranean): progress and prospects

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The particular geographical position of Tunisia (North Africa), between the western basin and the eastern basin of the Mediterranean Sea, its proximity to the Sicilian Channel, the intensification of maritime traffic, aquaculture facilities, anthropic pressures (overfishing, pollution, illegal

fishing, etc.), and global warming explain the increasing flow of non-indigenous marine species (NIS) into Tunisian waters. To date, about 160 marine NIS have been inventoried in this area, and some of them, defined as invasive alien species (IAS), are a real threat at the national and regional levels. Currently, monitoring and control of marine NIS, and particularly marine IAS areas, are of national priority. Moreover, a strategic framework for the prevention and management of marine IAS is being prepared. The identification of these species is a necessary prerequisite for bioinvasion surveys and the conservation of biodiversity in the context of global change. However, nowadays, taxonomists are increasingly rare, and identification keys are not always available, which would explain some misidentifications reported in the literature. For example, the blue crab *Portunus segnis* (a highly invasive species in Tunisia) has been identified for decades as *P. pelagicus*, and the confusion has been only recently corrected based on genetic analyses. Here, we present the progress in barcoding marine NIS in Tunisia. To date, only about half of marine NIS have DNA barcodes available in BOLD, and future efforts need to be focused on collecting and barcoding the remaining species. These concerted efforts across various Tunisian organizations will be vital in order to complete the database as a prerequisite for the wide application and implementation of DNA barcoding as a tool for rapid identification of Tunisian priority species.

Ancient sedimentary DNA reveals both climatic and human impact on vegetation history in Varanger, northern Norway

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Background: Integration of archeological evidences to ecological studies help assess human impact in vegetation development. Mortensnes (Ceavccageađge in Sami) is a well-investigated archaeological site in northern Norway. It is also close to the eastern colonization corridor of northern Norway, both in botanical and human terms, and is expected to provide a signal of early human interaction with vegetation in the past. **Results:** Using sedimentary ancient DNA from Nordvivatnet at Mortensnes, we reconstructed temporal changes in the plant species composition of vegetation in this area for the first time. We also analyzed the sediments for loss on ignition (LOI), and other nonbiological proxies. The sediment chronology covers the entire Holocene and part of the late Pleistocene. Our results show a relatively species-poor Late Glacial and Younger Dryas period, dominated by arctic taxa like *Dryas*, *Papaver*, and *Saxifraga oppositifolia*. The total plant species richness increased rapidly in the early Holocene, concurrent with increasing LOI, indicating a generally higher biological productivity. Nitrogen-demanding species such as *Anthriscus sylvestris*, *Chamerion angustifolium*, and *Filipendula ulmaria*, likely indicating anthropogenic activity in the vicinity of the lake, appear from around 11 000 cal. a BP, the same time as the oldest dated archeological records from Mortensnes. The occurrence of pioneer species indicative of past climatic episodes suggests that the historical climate strongly affected vegetation in northern Norway, particularly during the Late Glacial-early Holocene transition. During the Holocene, several ruderals and human activities appear concurrently, suggesting anthropogenic factors as an important driver of vegetation change despite the anticipated low human impact in this region. **Significance:** Linking sedimentary ancient DNA and archeological evidences, this study shows human impact as an important driver of compositional changes in vegetation in an otherwise anticipated low-human-impacted region and enhances our capacity to explore possible past interactions between humans and vegetation in the sub-Arctic.

Exposing an imprint of arctic climate change through the use of DNA barcodes

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Background: The climate of the Arctic is currently changing faster than that of most regions on the globe. To understand the resulting imprints, we need new tools for processing ecologically relevant samples collected over long-enough time or large-enough areas. Yet, most arctic organisms are small black dipterans and hymenopterans, and characterizing Arctic communities using morphological criteria is thus a path strewn with thorns. Here, DNA barcodes may offer the way forward. Relatively low species richness makes Arctic communities particularly amenable to highly resolved approaches based on DNA barcodes. **Results:** In this talk, I will use our most recent work to illustrate how we have used DNA barcode-based methods to characterize Arctic communities, and to resolve imprints of ongoing climate change. **Significance:** While differential responses to environmental changes is currently altering the structure of Arctic communities, morphological identification of ecologically relevant community material can only be achieved by a few expert taxonomists in the world. Since the ongoing changes are percolating to functional associations, e.g., between plants and their pollinators, we urgently need more time series from full communities and larger materials collected over expanded spatial scales. Our recent improvements in community characterization and quantification, coupled with recent and imminent gains in cost-efficiency, make approaches based on DNA barcodes an attractive option for use in Arctic community ecology and in applied biomonitoring.

From DNA-barcode libraries to global macroecology and macroevolutionary studies in insects

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Background: Insects are the most speciose group of terrestrial organisms and are strongly affected by global environmental and climatic changes. They exhibit a remarkable variety of forms and life history trait combinations not represented among vertebrates and are responsible for many ecosystem services and disservices. Yet, our knowledge of their diversity and distributions, as well as our understanding of their evolution and diversification dynamics through space and time, remains fragmentary. Two moth families, Saturniidae and Sphingidae, represent an unparalleled insect model to address this shortfall. This group comprises about 5000 species and is unique in being covered by DNA-barcode libraries representing more than 80 000 records in total and nearly 95% of all known species. **Results:** Here, we first present how we combined this DNA-barcode based assessment of species diversity with (*i*) a vast amount of additional occurrence records, (*ii*) a comprehensive phylogeny derived from genomic data (Ultra-Conserved Elements and RADSeq), and (*iii*) a broad documentation of their life histories. Through this holistic approach, we propose the first account of species richness patterns at a global scale in a group of insects whose diversity peaks in the intertropical region. Highlights in areas where patterns differ in these groups are given and discussed in the light of contrasted life histories. We also present the inferred diversification dynamics, in space and time, of *Copaxa* moths, a group of ~120 described species of saturniids that diversified in the Neotropics. **Significance:** Overall, we expect that our efforts will shed light on the processes governing the extant diversity of insects and help us understand how global changes will affect them, how they may or

may not adapt to these changes, and how best we can act to conserve the species and preserve their roles in our ecosystems.

Molecular food web analysis unravels how plant fertilization affects the stability of biological control

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Background: Biological control of pests is a key ecosystem service sustainable farming relies on. However, the level of biocontrol can fluctuate considerably due to farming practices, making the stability of this ecosystem service hard to predict, posing a hurdle to implementing biocontrol in farming. Hence, a better functional understanding of these systems is needed to understand how specific management actions drive the stability and resilience of biocontrol. Plant fertilization is a key measure in arable farming and has been shown to affect both resource availability and habitat conditions for arthropods. So far, we know little how fertilization types affect these processes and the dynamics in food webs. The latter, however, is at the heart for obtaining a better functional understanding of biocontrol of pests. Fortunately, recent advances in DNA-based techniques allow to employ an empirical food web approach and to quantify feeding links in complex ecological networks. **Results:** Using DNA-based gut content analysis of ~7000 generalist arthropod predators, we investigated how the consumption of functionally important prey such as aphids, springtails, and earthworms changed in response to fertilization type. This was realized in a replicated field experiment over two years including manure, compost, and conventional inorganic fertilizer in cereal fields in Tirol/Austria. Our findings demonstrate that organic fertilizers such as manure or compost not only provide additional food for decomposers, but that they contribute to restructure food webs, as alternative prey becomes more available for predators. We show how this occurs by coupling community data of both prey and predator taxa with trophic-interaction networks. This allowed us to describe how predator communities, occupied niche space, and diet preferences changed within predator communities in response to different fertilization types. **Significance:** Our findings demonstrate that the type of plant fertilization regulates food web dynamics and thereby affects the stability and resilience of biocontrol ecosystem services.

Tackling biological invasions and hybridization between native and non-native species by means of a multidisciplinary integrative approach

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Background: Tackling species introduction and hybridization between native and non-native species has been considered as a great concern. Early and accurate identification of non-native species in all developmental stages is required; however, morphological identification of ichthyoplankton might not be accurate. Extensive hybridization with non-native species was reported as a hidden threat to the native *Prochilodus hartii* in the Jequitinhonha River basin (southeast Brazil). To address this issue, we used a multidisciplinary approach combining DNA barcoding (650 bp of the mitochondrial gene COI), reproductive biological data (macroscopic and microscopic analyses of the gonads), and environmental DNA (eDNA) metabarcoding. **Results:** We obtained 81 samples from 5 locations. DNA barcoding allowed the detection of 13 species (48.2% of non-native species). Regarding *Prochilodus* spp., *P. hartii* was detected in 15.4% of eggs/larvae, and the non-native species *P. argenteus* and *P. costatus* were recovered in 69.2% and 15.4%, respectively. Haplotypes from *P. hartii*, *P. argenteus*, and *P. costatus* were recovered among the adult fish ($n = 63$), and incongruence between morphological and molecular identifications indicated the presence of hybrids. Specimens from all species (including putative hybrids) showed an advanced stage of gonadal maturation. eDNA metabarcoding allowed the detection of many non-native species; however, due to the low taxonomic resolution of the 12S fragment analysed, species of *Prochilodus* spp. could not be distinguished by this method. Additionally, identification of hybrids will be conducted based on SNP markers obtained by ddRADSeq (double-digest restriction site-associated DNA sequencing), and their potential to reproduce will be evaluated by comparing the molecular identification with reproductive data. **Significance:** These results provided the first evidence that the non-native species are reproducing in this basin and suggests that the hybridization process might be occurring in this basin since non-native and native species were sharing the same locality at spawning time.

The Neotropical biodiversity in the genomics and metagenomics era: unveiling the hidden diversity and potential threats to its conservation

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Background: Interactions of multiple anthropogenic stressors are having a great impact on Neotropical biodiversity. The trade-off between socioeconomic development and species/habitat conservation remains a hefty challenge in biodiversity-rich countries. Thus, management actions are likely more effective if underpinned by innovative and promising molecular tools (associated with traditional techniques). Genomics and metagenomics tools can greatly contribute to biodiversity assessment and conservation in remote and poorly accessible areas. We applied DNA barcoding (650 bp mitochondrial gene COI) and environmental DNA (eDNA) metabarcoding (172 bp mitochondrial gene 12S) to evaluate their potential in two Brazilian river basins. **Results:** DNA barcoding of the Doce River basin—which faced the worst ever environmental accident reported for South American catchments due to a mining collapse—allowed the accurate description of fish biodiversity pre-dating the environmental disaster. The results obtained stressed the occurrence of a hidden diversity, through the presence of cryptic species, species complex, or historical errors in morphological identification, calling for a more robust DNA-assisted cataloguing of biodiversity-rich ecosystems. In the Jequitinhonha River basin, we evaluated the potential of eDNA metabarcoding as a biodiversity assessment tool. Water and sediment samples obtained allowed the detection of 252 molecular operational taxonomic units (MOTUs), of which at least 34 were assigned to the species level, including new reports of putative native and introduced species for this basin. A baseline for eDNA studies in Brazilian rivers was also suggested by comparing different preservation methods, sampling media, and sampling times. **Significance:** The results obtained demonstrate that DNA barcoding and eDNA metabarcoding can significantly enhance our ability to catalogue fish biodiversity and assess its changes and the factors associated with such changes. While traditional sampling methods and morphological analysis will continue to be important for recording biodiversity, this DNA-assisted vision can speed up the process of data collection, clarify long-standing ambiguities, and flag areas for priority management.

Constructing a DNA barcode reference library for plants and mammals from Lebanon

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The Mediterranean Basin constitutes a biodiversity hotspot representing 0.3% of the world's biodiversity. Lebanon is considered to be an important site in this basin, with 9116 characterized species in total (4486 for fauna and 4630 for flora, of which 91 are endemic). Major gaps, however, exist for fungi, lichen, insects, bryophytes, and pteridophytes characterization. According to the BOLD database, only 344 taxon records with sequences are published, forming 151 BINs, and of these, 106 have species names. Biodiversity in major taxonomic groups is poor, sporadic, and understated within public platforms, and this is mainly due to the lack of funding needed to expand this taxonomy. Our team has joined the Barcode of Life initiative that has provided us with the opportunity to create our own reference library for mammals and plants in Lebanon, especially the endemic ones using DNA barcoding. Plant leaves were collected from the natural reserve of Horsh Ehden. For the mammal samples, DNA was taken from road kills or dead animal remains found in the forests or from museums. DNA from fresh material was extracted using standard protocols, while for museum samples, a DNA isolation protocol was applied. DNA was then amplified by PCR using the plastid markers rbcL and trnL (UAA) for plants and 12S for animals, then sequenced by the Sanger technique. Using these methods, we have published to date the sequences of 52 plant and 16 mammal species. DNA barcoding has become a universal molecular identification system of species with demonstrated reliability for at least a decade. Using barcoding for species identification will definitely help us understand the biodiversity of our country's ecosystems and expand our database applying this method on other taxonomic groups, such as insects and fungi in the near future.

Hidden diversity and deep divergence in a presumed broadly distributed polychaete along the Atlantic coast of the Americas

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Background: DNA barcode-based studies on the diversity of polychaete species occurring in Brazil have received little to no attention. Nevertheless, it has been suspected that several polychaete taxa have hidden diversity, including cryptic species. *Laeonereis culveri* (Nereididae) represents a case of taxonomic ambiguity, which since 1971 is assumed as a single species occurring from north to south along the Atlantic Coast of the Americas. In 2009, a taxonomic morphology-based revision corroborated the previous status. The current study aimed to review *L. culveri* taxonomy, diversity, and distribution through the morphological and DNA barcode-based examination of 10 populations spread along the American Atlantic coast from Massachusetts, USA, to Mar del Plata, Argentina. **Results:** Populations were confirmed as *L. culveri* based on external diagnostic characters. The COI barcodes split the morphospecies into six completely sorted molecular operational taxonomic units (MOTUs), displaying deep genetic divergence (ranging from 10.1% to 28.7%). All MOTUs were clearly geographically sorted, except for the overlap in the geographic boundaries between two MOTUs occurring in São Paulo state, Brazil. Preliminary results, using sequences from 16S rRNA and 28S rRNA genes, support the same six MOTUs and geographic sorting identical with the COI-based pattern. **Significance:** We have found strong evidence for the existence of a complex of at least six hidden species within the *L. culveri* morphotype. The role of *L. culveri* as an important bioindicator of organic enrichment in estuaries needs to be revised in light of these new findings, namely through detailed ecological characterization of the MOTUs here detected. The deep divergences and geographic sorting of these species suggests that their current distribution still reflects a diversification process that likely initiated millions of years ago. *L. culveri* may therefore provide an important case study to bring light into the poorly known phylogeographic history of marine invertebrates along the Atlantic coast of South America.

FASTFISH-ID™: a universal single-tube test for rapid DNA authentication of any species of commercial fish using a portable device

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Background: Species mislabeling/substitution threatens the economic welfare, safety, and sustainability of the seafood industry and harms brands and consumer trust. Conventional COI DNA barcoding is not suitable for routine species monitoring along supply chains because of slow and costly off-site sequencing. FASTFISH-ID™ is a revolutionary and cost-effective DNA barcoding test that empowers anyone anywhere along the supply chain to rapidly authenticate any species of commercial fish in just 2 h in a simple "sample-in, answer-out" portable device without sequencing using the same set of reagents. FASTFISH-ID™ integrates seamlessly into existing fish DNA barcoding protocols: FASTFISH-ID™'s specially designed hybridization probes convert informative sequence regions within the complete amplified COI DNA barcode into highly reliable species-specific fluorescent signatures that are then referenced to an expanding cloud-based database for species authentication in seconds without opening the reaction tube. If needed, mislabeled and unknown samples can be directly sequenced for regulatory compliance or for reference library addition, respectively. Sample preparation is also fast: samples are just disrupted in a lysis reagent and then diluted into the reaction tube for immediate authentication, regardless of starting DNA amounts. FASTFISH-ID™ eliminates the need for separate species tests or to send samples out routinely for sequencing. **Results:** FASTFISH-ID™ was validated by two independent laboratories in the UK using a blinded panel of 18 commercial fish species comprised of 1–8 biological replicates (three technical replicates each) with 96%–100% success. **Significance:** FASTFISH-ID™'s turnkey "sample-in, answer-out" operation, portability, and cloud-based analysis software makes authentication of any fish species easier, faster, and cost-effective. Rapid and convenient FASTFISH-ID™ species authentication will protect the seafood industry against the vulnerabilities of species substitution/mislabeling. The same technology can be used for analysis of virtually any group of animals, plants, or microbes on Earth by designing the appropriate PCR primers and probe sets covering numerous genera and species.

Salad within: a genetic survey of diet of the endangered New Mexico jumping mouse (*Zapus luteus luteus*)

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Background: The United States lists *Zapus luteus luteus* (formerly *Zapus hudsonius luteus*) under the Endangered Species Act, legislation that enacts recovery for species threatened by extinction. Sensitive to habitat loss, *Z. l. luteus* occupies high elevation (2000–2800 m), riparian zones in the southwestern United States. The species forages, mates, and raises young during the summer and hibernates for the rest of the year. Resource managers benefit from thorough dietary surveys to

guide habitat recovery, but the lack of data for *Z. l. luteus* limits flexibility in management direction. Studies of congenerics suggest frequent consumption of various plant species (seeds) and lower frequency of arthropods. **Results:** We used DNA metabarcoding to target potential plant and arthropod dietary items in trap-collected feces from 2016 to 2018, assembling reference libraries from the Barcode of Life Data Systems (BOLD) for taxonomic classification. To date, we detected 89 plant genera for 82 individual jumping mice that we sampled across their geographic distribution. Richness of plant taxa significantly increased ($p < 0.05$) prior to hibernation, coincident with an average gain of 5 g in body mass. Detections of arthropods supported previous studies of congenerics. **Significance:** Attributed to the difficulty of field observation, only seven plant species were known as diet items for *Z. l. luteus* prior to our study. The number of potential diet items revealed by DNA barcodes is now approaching 100 genera. The diversity of detected plant taxa largely mirrors the composition of jumping mouse habitat and supports previous hypotheses that seed availability governs resource selection. Temporal patterns of dietary richness also suggest that phenological patterns of plants in their habitat may facilitate accumulation of hibernation weight. Our study presents the first detections of arthropods as potential diet items for *Z. l. luteus* but is unclear if arthropod taxa are selected or opportunistically consumed.

Assessing multi-taxon biodiversity in terrestrial ecosystem using environmental DNA: is soil sampling enough?

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Background: Environmental conservation, management, and biomonitoring require information on species distributions and abundance over different spatial/temporal scales. The high levels of terrestrial ecosystem biodiversity, essential for ecosystem function, are often attributed to their structural spatial complexity and interactions. Nevertheless, the data used to draw terrestrial biodiversity conclusions often involves a single soil sampling method, overlooking the multiple biological aboveground components. Still, it is not known how much biodiversity the aboveground habitats contain and can add to biodiversity studies when compared to soil. Therefore, the aim of this study is to yield unbiased, representative samples of multi-taxon biodiversity, enabling comparison across terrestrial ecosystems. To address this knowledge gap, we thoroughly sampled 10 Danish nature sites on a gradient of increasing structural complexity ranging from simple agricultural fields over grass/shrub to old-growth forests. Eight different microhabitats were selected for sampling, when locally present. Amplicon sequencing was performed with primers targeting groups such as fungi, insects, and arthropods. **Results:** By analysing species accumulation, we provide evidence that biodiversity assessments based solely on soil sampling does not represent the diversity within the three dimensional most complex habitats. Aboveground biodiversity contributes with more unique biodiversity with increasing ecosystem complexity. Few exact sequence variants (ESVs) were shared between all the microhabitats, demonstrating that the communities assessed are spatially restricted in terrestrial ecosystems. The additional sampling of litter and coarse deadwood enhanced the biodiversity of more complex habitats, having a complementary role for the biodiversity. **Significance:** Small-scale heterogeneity can hamper the detection of many species and ecosystem processes that are dynamic over short time scales (i.e., minutes to days), or that respond to fine-scale environmental variation, such as insects. The understanding of the small-scale community's distribution can narrow down the factors driving community structure patterns and link that structure to observed heterogeneity in biogeochemical processes.

Genetic variability and population connectivity of *Pinna nobilis* in the eastern Mediterranean Sea

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Background: The fan mussel *Pinna nobilis* Linnaeus, 1758 is an endemic species of the Mediterranean Sea included in the list of endangered species (Annex IV of the Habitat Directive and Annex II of the Barcelona Convention). It is one of the largest bivalves in the world, living partially buried in bare sandy substrate or in seagrass meadows. The assessment of genetic variation of *P. nobilis* and the evaluation of connectivity among the different populations are important elements for the conservation of this species. **Results:** For this purpose, various genetic markers were chosen in order to depict the levels of genetic variability between three populations of *P. nobilis* in the eastern Mediterranean. Samples were collected using minimum invasion and nonlethal methods, to avoid stress induction in the individuals. DNA was extracted, followed by PCR amplification and sequencing of the selected barcoding genes, which were carried out to reveal the patterns of genetic differentiation at the population level. **Significance:** The results of this work will shed light on the distribution of the populations of *P. nobilis* and on the degree of connectivity between the different sampling locations. In addition, the results will unravel possible relationships of the populations to environmental variables, which could be used as a proxy for monitoring the population distribution and ecosystem resilience.

Crosslinking, coverage, and access improvements in global biodiversity data

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The world's digital biodiversity evidence compiled through GBIF (the Global Biodiversity Information Facility) has been dominated by data from citizen science and natural history collections. The next frontiers in data integration lie in quantitative ecology and molecular biodiversity. GBIF partners with UNITE, a data management and sequence identification environment for fungal barcode sequences with >73 000 species hypotheses derived from >800 000 sequences. Inclusion of stable, DOI-trackable operational taxonomic units (OTUs) and Linnaean names in UNITE into the GBIF taxonomic backbone enables indexing of fungal metabarcoding data worldwide as GBIF occurrence and event data (e.g., BIOWIDE). Building on this foundation and partnerships with BOLD and SILVA, we are extending GBIF's ability to aggregate molecular data. GBIF and the European Bioinformatics Institute (EMBL-EBI), supported by ELIXIR, are extending their collaboration to share molecular species occurrences. This collaboration adds a significant molecular data stream to GBIF.org (>7.7M records to date), bridging the gap between biodiversity studies based on molecular data and those that rely on morphological data. Moreover, GBIF will stream amplicon and 'omics data from MGnify as standardized Darwin Core sampling-event datasets. Publication of sequence-based occurrence records from EMBL-EBI via MGnify and ENA increases the representation

of microscopic diversity and reduces the well-known taxonomic bias in favour of easily identifiable taxa and against cryptic biodiversity. By combining morphological and molecular biodiversity in a single taxonomic backbone using Linnaean names, OTU codes, and molecular occurrence data from BOLD, UNITE, EMBL-EBI, and SILVA, the GBIF network will address major spatial, temporal, and taxonomic biases while supporting scientific efforts to understand functional biodiversity. GBIF promotes open data while protecting data provenance through data citation, and new API-based tools will further scale up cross-platform data integration and data indexing. These efforts address the growing need for easily accessible molecular biodiversity evidence.

Effects of climate-induced tree-dieback on freshwater and Malaise trap communities in the Bavarian Forest National Park

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Background: Mountain forest ecosystems are under increasing pressures. Rising global temperatures lead to increased frequency of droughts and pathogen outbreaks, which in turn lead to drastic changes in forest structure and widespread die-off of key tree species. How these forest die-offs affect arthropod communities and biodiversity overall is not well understood, and current biomonitoring methods are inadequate for large-scale quality assessments. The Bavarian Forest National Park has a long history of research on mountain forests, in particular on spruce (*Picea abies*), and an ongoing insect monitoring program. **Results:** We used Malaise traps to sample invertebrates in 30 plots within three different forest habitats (intact, naturally disturbed, salvage-logged) along an elevational gradient over four months in summer 2017 for a total of 240 samples. We used kick-netting to sample invertebrates in 30 stream sites within the same forest habitats. DNA was extracted from subsamples and metabarcode libraries (313 bp COI) were sequenced with Illumina MiSeq. We used replicate PCRs ($n = 3$) and twin-tagging, together with a mock community and negative controls, in order carry out stringent filtering. To date, >4000 operational taxonomic units (OTUs) have been identified for the Malaise traps, with >70% belonging to Diptera or Hymenoptera and Ichneumonidae being the most diverse family (>800 OTUs). **Significance:** The project is part of a larger effort that combines ecological and socio-economic approaches to understand mountain forest die-back and its implications for biodiversity. Our specific goal is to develop a fast, repeatable, and reliable method for biomonitoring of terrestrial and aquatic invertebrates in mountain forests that will help practitioners such as national parks and forest owners. We hope to further our knowledge of methodological processes and techniques to make monitoring results in the future more comparable and standardized, and to gain additional insights into the ecology of invertebrate communities in mountain forest habitats.

Multiplexed chloroplast and nuclear marker sets for differentiation of 19 relevant poplar species for breeding

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Background: The genus *Populus* includes about 30 species classified in six sections, where some are cross-compatible even between sections. Therefore, many naturally and additionally, due to breeding programs, artificially produced hybrids exist, often without any information about involved species. Due to the very high variability of *Populus* hybrids, species identification using morphological characters is sometimes difficult. For this reason, we combined classical barcoding markers with newly designed chloroplast and nuclear markers with the aim to develop sets of markers for the differentiation of the 19 most widely used poplar species. **Results:** We used 1–32 individuals per species for sequencing of a total of nine chloroplast and four nuclear regions. Overall, we found species-specific SNPs or indels for 14 of the 19 species in chloroplast and 17 out of 19 species in nuclear regions. Nucleotide diversity in the analysed regions varied among species and was highest for the three species of the section *Populus* (*P. alba*, *P. tremula*, *P. tremuloides*) followed by the Aigeiros species *P. nigra*. We developed methods to identify species by either species-specific nucleotide variations or, without initial information for the species, by using markers either in a step-wise procedure of exclusion or in a multiplexed marker set. The two species *P. koreana* and *P. ussuriensis* are not distinguishable by applying both procedures. **Significance:** Hybrids between various *Populus* species belonging to the same or different sections are commonly used in short rotation coppices for biomass production because of their superior growth and advanced resistance traits. We present a comprehensive study on the identification of a high number of *Populus* species that, to our knowledge, have not been performed across such a wide range and with feasibility in any laboratory.

Barcoding Canada's ecozones: past progress, future steps

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DNA barcode reference libraries are the foundation for scalable, high-resolution biosurveillance systems. Over the past 15 years, 2.1 million Canadian specimens have been barcoded, providing coverage for 77 391 BINs and 31 253 named species. Most of these records (95.2%) derive from terrestrial settings; just 4.8% derive from the marine environment although it comprises more than a third of Canadian territory. The differences are even more profound when one considers coverage from an ecozone perspective. Coverage for the 15 terrestrial ecozones averages 129 909 records but ranges from 2463 records (Taiga Shield) to 582 663 (Mixed Wood Plain). Coverage for the 12 marine ecozones is far lower, averaging 5591 records and ranging from a low of just 58 (Arctic Archipelago) to a high of 22 617 (Strait of Georgia). The average number of records on an areal basis differs by more than an order of magnitude between marine and terrestrial settings (1.2 vs. 19.5 records per 100 km^2). When viewed at a species level, coverage for marine phyla ranges from near zero for bryozoans, ctenophores, and nematodes to 56.6% for arthropods. Moreover, many species in the oceans await discovery as metabarcoding studies on marine benthos indicate high diversity. Studies are now underway to strengthen coverage in marine environments through studies on hyperdiverse groups such as harpacticoid copepods and nematodes that have seen little prior investigation.

Identification of tree species in wood composite products by DNA barcoding

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Background: In Europe and in the USA, the detailed labeling of imported wood is an attempt to control illegal logging. Finding out the mixture of species in wood composite products poses a great challenge due to the use of different wood species in one product; the exposure of wood to chemicals, heat, and pressure during the production process; and the restricted applicability of wood anatomical methods. **Results:** Wood composite products often consist of wood from several tree species, which may belong to the group of angiosperms as well as to the gymnosperms. We are developing a set of genetic markers for the differentiation of kinds of frequently used wood on different taxonomic levels. The combination enables the development of an application protocol for an individualized and minimized marker set for each genus and species of interest. The marker development is based on a genome-wide identification of SNPs and InDels in chloroplast and mitochondrial genomes. For the differ-

entiation of angio- and gymnosperms, a CAPS marker was developed based on a SNP identified in the mitochondrial COX1 gene, which is used for species identification in many animal groups. Additionally, the absence or presence of certain ndh genes in chloroplast genomes will be used to confirm these results. At the genus level, e.g., in the family of Betulaceae, species belonging to the genus *Betula* can be separated from all others by only one CAPS marker in the matK gene, a formerly suggested chloroplast barcoding region. In a next step, we will also include gene distance and intron length information in the search for new markers. **Significance:** Illegal logging is the most profitable natural resource crime in the world. Thus, the DNA-based analysis of wood samples plays an important role as a law-enforcement tool in the determination of botanical species and geographic origin.

Developing PCR-RFLP and SNP-based markers for determining cytoplasmic male sterile factors in the genus *Amaranthus* (Amaranthaceae)

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Background: *Amaranthus* L. is an herbaceous plant comprising approximately 70 species, with three subgenera, which contains both cultivated and wild types. The cultivated species are used for food grains, leafy vegetables, potential forages, and ornamentals. Genetic diversity analysis in amaranths is important for the development of a core set of germplasm from widely diverse populations and for the effective utilization of plant genetic resources. Cytoplasmic male sterility (CMS) is one of the most important traits in crop breeding, which has been used for commercial seed production by F1 hybrid cultivars of *Amaranthus* species. Our aim is to develop a polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) marker to distinguish male-fertile (N) and male-sterile (S) cytoplasm in *Amaranthus* species by examining the distribution of the haplotypes in diverse breeding lines, cultivars, and wild. **Results:** The PCR-RFLP marker was located in a chloroplast psbA gene amplicon. Digesting the amplicons from different cytoplasm (either N/S)-containing varieties with restriction enzyme revealed and distinguished the N and S with functional and substitution cytoplasmic site. The developed PCR-RFLP marker was validated for cytoplasmic male sterile factors in 15 samples belonging to the wild and cultivated cultivars, which showed CMS-specific sequence-characterized amplified region (SCAR) marker. Moreover, the PCR-RFLP marker can identify N- or S-cytoplasms in DNA sample mixtures in which they are 10-fold less, indicating that use of the marker has diagnostic precision. **Significance:** We also confirmed the efficacy of the SNP detected in the psbA gene for high-throughput discrimination of CMS factors using real-time PCR. This approach is useful for the identification of CMS factors in large *Amaranthus* breeding populations and also for facilitating the crop's improvement.

DNA barcoding of Norwegian forest Oribatida — preliminary results reveal several taxonomic problems

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Background: Oribatid mites (Sarcoptiformes) are a very common, abundant, and diverse group with important roles in ecosystems and are also known as good bioindicators. Worldwide, Oribatida (excluding Astigmata) are represented by 9400 named species and 161 families. The Norwegian checklist mentions 244 species. BOLD includes over 43 000 specimen records, 40 000 sequences, and about 50% of all world-known families represented. The public data include 27 000 sequences in 2300 BINs. However, the number of named species is low, seriously limiting the use of this database for the identification purposes. The public BOLD database includes 185 named oribatid species only (i.e., 2% of all oribatid species known worldwide). **Results:** During ongoing studies on the diversity of Oribatida in Norwegian broad-leaved forests, 272 sequences (80% of barcoded specimens) in 91 BINs were obtained, representing 86 species, 53 genera, and 34 families. Only 20% of the species were represented in BOLD. Most species were monophyletic, with limited mitochondrial DNA variation. However, in about 10% of them the variation was 5%–10%, and in a further 10% it exceeded 10%. High mitochondrial DNA variation was noticed in species with a semi-cosmopolitan distribution and in some less-studied taxa. **Significance:** These preliminary results reveal 17 taxa that require special attention, as they might represent species complexes and include cryptic species. We also contributed 60 named species of Oribatida to the BOLD database.

Molecular diversity in the monotypic catfish genus *Mastiglanis* (Bockmann, 1994)

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Background: Catfish of the Family Heptapteridae are small-to-medium sized benthic omnivores, generally found in smaller streams, and endemic to the Neotropical region. Many heptapterids present systematic challenges because of the morphological similarity between species and even genera. The monotypic genus *Mastiglanis* is frequently identified incorrectly as *Imparfinis* in regional ecological surveys because of the similarity in terms of long barbels, a deeply forked tail, and limited pigmentation of the body. The genera differ most obviously in terms of pectoral fin ray lengths, and this character can often be lost during rough sample handling or naturally, prior to collection, through the action of predators from which the individual has narrowly escaped. We therefore applied a DNA barcoding approach to assess the potential existence of divergent lineages that may represent overlooked or cryptic species. We bidirectionally sequenced the COI-5P fragment (~650 bp) commonly used in vertebrate taxa and shown to be useful for species delimitation in freshwater and marine fish taxa. **Results:** Sequences were produced for 17 individuals identified as *Mastiglanis asopos* from nine different localities in the eastern Amazon—Pará state, Brazil. These sequences were combined with public data for other species in the family to produce a phylogenetic tree. These data were then analyzed using both threshold and coalescent species delimitation techniques. The overall phylogeny confirmed that *Imparfinis* and *Mastiglanis* are distinct evolutionary lineages, but showed that the samples identified as *Mastiglanis* do not form a monophyletic group. They represent two very divergent clades (probably separate genera), one of which contains five distinct lineages. **Significance:** The genus *Mastiglanis* is described to have a very large distribution across cis-Andean drainages of South America. As such, further sampling is required to determine the geographic distributions of each of its lineages and incorporate the information into integrated systematic results.

Spatio-temporal dynamics of lotic eDNA-derived metacommunities

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Background: Accurately measuring biodiversity is essential for ecology, molecular ecology, and environmental monitoring. Recently, en-

vironmental DNA (eDNA)-based sampling and metabarcode-based species identification have been shown to increase sampling accuracy while cutting cost and time compared to traditional methods. While several prominent studies have shown several eDNA-based sampling can detect a wide range of diversity, studies are limited in their spatial, temporal, and environmental conditions to field validate the eDNA-based methods in loctic environments. **Results:** We collected aquatic eDNA samples from 14 sites along a 35 km stretch of the Conwy River from 19 time points from April 2017 to April 2018 (~900 total samples). Additionally, we took monthly macroinvertebrate kick-net and chironomid exuvia samples from the Conwy Reservoir, at the head of the River Conwy. Metabarcode amplification was performed using four barcode primer sets (COI, 18S, 12S, and rbcL). All library preparations were done at Bangor University using a Gilson Pipetmax robotic platform. Library preparations were handed over to the University of Birmingham to be quality checked and normalized prior to submission to HiSeq sequencing (4 lanes in total). Romain Derelle has started to bioinformatically sort the first Hiseq lane results using a novel pipeline (Broccoli), and Mat Seymour will be assessing the metacommunity dynamic using mixed-effect models. **Significance:** Our study represents one of the most ambitious lotic eDNA studies and will be one of the largest single eDNA datasets. The transport dynamics of eDNA-derived communities is still largely unknown, and these findings will clarify the spatio-temporal dynamics of eDNA in lotic environments. While these data will initially be used to answer relatively basic, but essential, questions regarding the spatial and temporal dynamics of lotic eDNA, they will also allow for a wide range of ecological, bioinformatics, and molecular questions to be assessed and explored further.

Medicinal Materials DNA Barcode Database (MMDBD) for molecular authentication of medicinal herbs and a pilot assessment of annotation quality and taxonomic reliability of DNA barcode sequences

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Background: Authentication of medicinal materials by DNA technology is gaining popularity in the herbal industry. In 2010, our team created the Medicinal Materials DNA Barcode Database (MMDBD) version 1.0, an interactive database of DNA barcode sequences of medicinal materials obtained by our team as well as downloaded from GenBank. GenBank has long been known to contain sequences that are inaccurate, poorly annotated, and(or) taxonomically incorrect/mis-identified. It was estimated that about 20% of fungal internal transcribed spacer sequences were insufficiently annotated and(or) mis-identified. Accurate and taxonomically reliable sequences are vital for correct species identification by DNA barcoding. **Results:** We have recently updated our database to MMDBD, making it a one-stop platform for BLAST, alignment, and primer design. A BLAST-based engine, Clustal Omega alignment tool, and Primer3 were incorporated to the web interface to allow easy sequence similarity search and alignment, as well as primer design. A pilot study has been carried out to evaluate sequences of DNA barcodes (rbcL and matK) and supplementary barcodes (trnH–psbA, trnL–trnF intergenic spacer, ITS1, and ITS2) of medicinal *Dendrobium* species downloaded from GenBank. Annotation integrity, sequence quality, and estimated taxonomic reliability has been assessed. Sequences evaluated were rated to different levels of reliability based on their annotation information and sequence quality. **Significance:** This is the first attempt to assess the reliability of sequences of DNA barcodes of medicinal herbs. Sequences rated to different levels of reliability will be included in our database, which will be useful for the scientific community and the botanical industry for species identification.

Authentication of Chinese traditional medicines Gusuibu based on DNA barcoding markers and SCAR markers from chloroplast genomes

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Background: Gusuibu is a traditional Chinese medicine for the treatment of bone diseases. In the Pharmacopoeia of the People's Republic of China, only the dried rhizome of *Drynaria roosii* is listed as the origin of Gusuibu. However, many Gusuibu adulterants with similarity in morphology, including *D. sinaca*, *D. bonii*, *D. delavyi*, *D. quercifolia*, *D. propinqua*, and *Phymatosorus cuspidatus*, are widely used in China. DNA-based markers need to be developed to efficiently distinguish the authentic Gusuibu from adulterants. **Results:** Twenty-two chloroplast genomes from seven species, including *D. roosii* and six Gushuibu adulterant species, were sequenced using the Illumina Hiseq 2000 platform. The cp genomes of *D. roosii*, *D. sinaca*, *D. bonii*, *D. delavyi*, *D. quercifolia*, *D. propinqua*, and *P. cuspidatus* were 154 181, 151 711, 151 542, 151 709, 151 570, 152 442, and 151 466 bp in length, respectively. Phylogenetic analysis indicated that cp genomes were able to distinguish *D. roosii* from adulterants and among adulterants. By comparing the cp genomes, highly divergent regions in cp genomes were identified, and one DNA barcoding marker and four sequence characterized amplified region (SCAR) markers were developed, and related universal primers were designed. **Significance:** The universal primers for DNA barcoding marker and SCAR markers amplification designed in the present study will be useful for economically and effectively distinguishing *D. roosii* from adulterants, and for guaranteeing the quality, safety, and effectiveness of Gusuibu herbs.

The use of DNA barcoding to monitor the species composition of *Pheretima* (Dilong) in herbal markets and their medicinal products

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Background: *Pheretima*, also called Dilong, is one of the famous traditional Chinese medicines usually used to treat thrombosis-related disease. According to Chinese pharmacopoeia, *Pheretima* is the dry earthworm body of *Pheretima aspergillum*, *P. vulgaris*, *P. guillelmi*, or *P. pectinifera* with the removing of viscera during the processing procedure. So obviously, it is difficult to identify the earthworm by traditional morphological methods because of the lack of morphological features that can be used for identification. **Results:** First, a DNA barcode reference library containing 127 species has been constructed through the collection of the common earthworms in China. Then, authenticity testing was implemented for 176 *Pheretima* herbal materials from herbal markets. The results showed that in addition to the species specified in the Pharmacopoeia, there is a large number of other species. Next, we used high-throughput sequencing technology to analyze earthworm species composition in 12 pack of medicine products containing Dilong. Successful sequencing obtained 6G data for each herbal product and assembled to about 112 full-length COI sequences. The average length of these full-length COI sequences is 1.5K and well solved the limitations of using short primers to amplify COI sequences in other DNA-metabarcoding studies. Finally, we found very rich earthworm species in herbal products and that were highly consistent with our survey. **Significance:** These findings suggest that half of the specimens were not identified as legal species, which truly

meant we should pay more attention to the supervision of *Pheretima* in the markets. And, this study further demostrated DNA barcoding is a very effective tool to identify earthworms, not only for ecologists, but also for herbal product regulators.

Overview of the outcomes of UN Biodiversity Conference 2018 and Q&A in relation to DNA barcoding

Junko Shimura

UN Secretariat of the CBD, Canada.

Parties, other Governments, and relevant organizations to the Convention on Biological Diversity meet every two years to consider the implementation of the Convention and its Protocols. In November 2018, the UN Conference made decisions to request the Secretariat to further promote and facilitate training on DNA barcoding. In a newly adopted supplementary voluntary guidance on invasive alien species, States are encouraged to use DNA barcoding as a tool to identify invasive alien species. The discussion on a post-2020 biodiversity framework was initiated, and a series of consultation meetings will take place this year to collect views on the next 10 years of strategies, which may enable Parties to take evidence-based actions towards life in harmony with nature. To this end, participation of the International Barcode of Life as a scientific body holding species and genetic biodiversity information and associated knowledges is welcome to join the process. Access to and use of digital sequence information on genetic resources (DSI) are recognized as contributing to scientific research, and an Ad Hoc Technical Expert Group was established to compile and synthesise information and views on the DSI, which will feed into the post-2020 biodiversity framework development. At the occasion of the International Barcode of Life Conference, the Secretariat will provide casual question and answer time on the above outcomes within the context of DNA barcoding and its related capacity-building, such as GTI-DNA-tech.

A thousand shades of grey: DNA barcoding the diverse, yet similar, *Rasbora* species (Ostariophysii, Cypriniformes, Cyprinidae) of Sundaland

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Background: Among the four biodiversity hotspots observed in Southeast Asia, Sundaland is the largest. Including the islands of Java, Bali, Sumatra, and Borneo, it hosts 800 species of the 1200 known freshwater fish species of Indonesia. This extreme diversity is explained by the complex geological and paleoecological history of Sundaland, resulting from plate tectonics and eustatic fluctuations during Milankowitch cycles. The Sundaland ichthyofauna is dominated by primary freshwater lineages, which colonized the area through the land bridges that repeatedly connected Sundaland to the Asian continent during glacial maxima. Currently encompassing 87 species distributed throughout Asia, *Rasbora* species are extremely diversified in Sundaland as nearly half of the known species occur there. The taxonomy of *Rasbora* species is problematic due to their extreme morphological similarity, their small size, and the complex taxonomic history associated with many of the oldest species descriptions. Our objective is to present the result of a collaborative effort to DNA barcode all *Rasbora* species of Sundaland. **Results:** A total of 1000 DNA barcodes for nearly 60 species of *Rasbora* and closely related genera (*Trigonopoma*, *Trigonostigma*, *Brevibora*, and *Pectenocypris*) were gathered. We analyzed samples collected at known type localities in Sundaland and used four species delimitation methods (BIN, GMYC, PTP, ABGD) to examine species boundaries and delimit operational taxonomic units (OTU). Our results show that *Rasbora* diversity is largely underestimated as OTUs amount to twice the number of nominal species, with unexpectedly high levels of genetic divergence (2%–4%). **Significance:** By incorporating DNA barcodes from known type localities in Sundaland, we were able to assemble the first reference library available to date for *Rasbora* and allied genera. While *Rasbora* constitutes a meaningful model for evolutionary studies in the area, the complexity of its taxonomy has constituted a major brake. The present study opens new perspectives on that front.

Comparing metagenomic and metabarcoding approaches in water samples from a marine environment

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Background: Analyzing environmental DNA (eDNA) is becoming a popular technique for environmental assessment activities thanks to the large amount of biodiversity data it generates relative to conventional approaches. The common methodology for eDNA-based assessments is metabarcoding, which involves targeted amplification of standardized gene regions (e.g., COI or 18S rRNA), sequencing the amplicons, and then mapping them against reference databases. Alternatively, metagenomics involves sequencing the entire DNA content of an environmental sample such as water. We set out to determine if the metagenomics technique was applicable to biodiversity analysis for environmental assessment, especially within the context of the extremely deep sequencing available with modern instruments. **Results:** We obtained marine water samples from Conception Bay South in Newfoundland and subjected these samples to both a COI metabarcoding and a metagenomics approach using an ultra-high capacity sequencing instrument, the Illumina NovaSeq 6000. In our preliminary study, 15.7M and 67.3M paired-end reads were analyzed in the metabarcoding and metagenomics studies, respectively. Taxonomy assignment was performed using Kraken2 against the nt database with a confidence cut-off of 0.95. We found that 4.7% of the metabarcoding reads could be classified, the vast majority of which (98.7%) were from the Eukaryotic domain. Conversely, only 1.08% of the metagenomics reads could be classified, and the majority of these (81.4%) were from the Bacterial domain. **Significance:** Our results indicate that only a very small fraction of metagenomics data can be classified, mostly bacteria. This is likely a reflection of both the actual biomass present as well as biases in the reference database—since there are many more completely sequenced bacterial genomes than eukaryotic genomes, random genomic DNA fragments from the environment are much more likely to find a match among the bacteria. We therefore conclude that in most environmental studies metabarcoding will produce data with greater utility than metagenomics.

Differentiation, identification, and phylogenetic relationships of methyl salicylate producing birch species through chloroplast and nuclear markers

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Background: The genus *Betula* is the largest group of ecologically and economically dominant woody plant species ("birches") in the subalpine climate zone. Birches have an extensive history of hybridization

and introgression that create obstacles to comprehend their taxonomical status and interspecific relationships. Additionally, birches are a rich source of anti-inflammatory methyl salicylate (MeSA) that is exploited in many drugs as an ointment for the treatment of muscle injuries and joint pain. It is the first study where multiple chloroplast and nuclear regions were employed to evaluate their potential applicability to determine the interspecific relationships of high and low MeSA-producing birches and to develop screening strategies through a molecular genetic approach. **Results:** Molecular analyses exhibited a high number of variations in nuclear gene regions, whereas chloroplast intergenic spacers, compared to coding regions, carry a considerable number of single nucleotide polymorphisms (SNPs) and displayed insights into the genetic architecture of birches. Additionally, the official barcoding loci displayed average (matK) and no SNP variability (rbcL) in the birch genus. Sequencing of chloroplast and nuclear regions resulted in 25 and 33 SNPs, respectively, in *B. lenta*, *B. alleghaniensis*, *B. maximowicziana*, *B. medwedwii*, and *B. nana* that could be exploited to develop rapid molecular test methods to reduce the cost of sequencing for breeding and selection. Phylogenetic analyses conducted by considering all generated sequences deepened the understanding of relationships between high and low MeSA-producing birches. The high MeSA-producing *B. lenta*, *B. grossa*, and *B. alleghaniensis* form the oldest clade in phylogenetic and network analysis, suggesting that the MeSA trait was likely initially present in the genus and has been lost several times during evolution. **Significance:** Considering possible ambiguity within the chloroplast and nuclear datasets, phylogenetic studies manifested that the MeSA trait is distributed in the genus *Betula* irrespective of their ploidy status.

DNA metabarcoding to quantify the ecological impact of forest decline on flying insect diversity in the Pyrenees

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Background: Forests suffer from an increase in frequency and severity of summer droughts and infestations of pathogens and insects. Those factors cause high mortality of some keystone tree species (forest die-offs). Yet, how tree mortality and associated changes in forest composition will affect local diversity and ecosystem functions remains unknown. Here, we aim at quantifying the impact of climate-induced forest decline on biodiversity by measuring changes in the taxonomic structure of invertebrate communities along silver fir (*Abies alba*) dieback and salvage logging gradients in the French Pyrenees. We examine patterns of variation in species diversity of flying insect assemblages collected by Malaise traps deployed in 57 silver fir-dominated experimental plots (one Malaise trap per plot) in the central and eastern Pyrenees. Sampling was carried out each month for over 4 months (May–August 2017). Samples were sequenced using Illumina MiSeq and analyzed using the DAMe twin-tagging pipeline approach. **Results:** We obtained 224 bulk samples filled with a solution of monopropylene glycol plus ethanol. Despite high levels of DNA degradation detected in our samples, we found no major impact on species detection, with more than 3500 operational taxonomic units (OTUs) in 18 different insect orders recovered. We found large species temporal turnover (Jaccard Index: May–August = 0.35), as well as changes in community composition but no significant loss of species diversity along the forest decline gradient. **Significance:** There is an urgent need to obtain detailed baseline data on species assemblages to quantify the impacts of climate change. Our study assessed biodiversity patterns on a scale and with a resolution that was previously impossible and provides data essential for evaluating future biotic change. Our workflow coupling metabarcoding and Malaise trapping is simple to use and provides an affordable, reliable, and verifiable way of monitoring forest biodiversity at a large geographical scale.

Assessing diversity of marine bivalves (Mollusca, Bivalvia) in Norwegian waters using DNA barcoding, with emphasis on Pectinidae

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Background: Pectinidae (Bivalvia) consists of more than 300 species. Twelve of them are recorded from Norway, although little work has been done on them. These 12 species have in this study been examined in detail, to make proper descriptions, as well as an identification key. Descriptions will be based on morphology, but species diversity will also be assessed with DNA barcoding. Together with DNA barcodes and morphology, species delimitation will contribute to a more reliable identification of all bivalves, including juveniles. Identification of small bivalves, species that never grow big, as well as juveniles of larger species, can be hard when using morphological characters alone. Pectinids are included in a large-scale effort to DNA barcode marine bivalves as part of the Norwegian Barcode of Life (NorBOL) project. In a reference library for Norwegian bivalves, 190 specimens of marine bivalves have so far been barcoded, mainly from Central Norway. 43 of these specimens are pectinids, which will be used for species delimitation of the Norwegian species in this family. **Results:** Out of 190 bivalve specimens, 82 have been sequenced with success; among these are 14 pectinids representing 6 species. From these pectinid specimens, along with sequences from GenBank, species delimitation will be done to examine how many species of pectinids there are in Norwegian waters. **Significance:** A reference library for Norwegian bivalves, with focus on Pectinidae, is important. Marine bivalves are an important ecological group and are central in environmental monitoring and assessments. Assessing species identity with DNA barcodes will help identification of small bivalves, including juveniles of larger species. Since many pectinids are commercial species, a good reference library for barcoding can aid in the finding of population structure even for small individuals. Barcoding can also be used to check for introductions of new species in Norway.

Population genomics and phylogeography of the African lion (*Panthera leo*) in Tanzania: a continental and country-wide genetic assessment

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Background: The African lion (*Panthera leo*) is listed as Vulnerable by the IUCN Red List, mainly threatened by prey base depletion and indiscriminate killing, as a result of retaliatory or pre-emptive killing to protect human life and livestock. Additionally, habitat loss by land degradation and conversion has led to the isolation of subpopulations, potentially decreasing gene flow resulting from weakened

connectivity and leading to genetic drift. In the present study, we investigated the evolutionary history and structure of the species at different spatiotemporal scales. **Results:** The mitochondrial cytochrome b gene (n = 128), 11 microsatellites (n = 103), and 9103 SNPs (n = 66) were investigated, including a large sampling from Tanzania, since it hosts the largest lion population among all African lion range countries. In addition to supporting the dichotomic continental-scale structure (i.e., West-Central vs. East-Southern, which is also found in many other savanna mammals with large distribution ranges), three lion clusters could be identified in Tanzania. The clusters are geographically distributed in the northern, southern, and western regions of the country. The genetic differentiation between these clusters appears to have resulted from the combined effects of recent anthropogenic pressure and environmental/climatic factors. **Significance:** The Tanzanian annual human population growth of 3.1% indicates that the human pressure on wildlife habitats is not expected to decrease in the short-term, resulting in an increased human encroachment on landscapes. Since male lions have relatively weak dispersal capabilities, and since the species barely co-exists with humans, further fragmentation of its habitat could lead to an even greater loss of connectivity between the mosaic of protected areas, which might lead to a rapid loss of genetic variability. Continuous monitoring of the identified strongholds would be highly recommended, especially since all three clusters have undergone recent demographic contraction, as supported by the census records.

Identification of disease vectors from foreign deployment sites of the Belgian armed forces using DNA-based technologies

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Background: Vector-borne diseases impact humans in almost every part of the world. Vector prevention/control measures help reduce the impact and spread of these diseases. In this framework, the Medical Component of the Belgian Armed Forces has launched a pilot project to investigate the culicid mosquitoes (Insecta, Diptera) at foreign sites where the Belgian Army is stationed, in order to better anticipate vector-borne disease threats during deployments. Indeed, besides potentially affecting the soldiers' health, diseases can compromise the mission. Surveillance of vectors on-site will allow determination of what, when, and if prevention/control measures should be implemented. **Results:** Adult mosquitoes were collected on military bases in Jordan, Gabon, and Mali during the surveillance phase. A comprehensive list of the target taxa occurring in each of these countries was established based on the available publications and reports. DNA sequences deposited in online reference databases (BOLD and GenBank) were evaluated for their usefulness in identifying vectors by DNA barcoding. Specimens (n = 178) were identified using DNA-based methods. Among these, *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, *Culex pipiens*, *Culex perexiguus*, and *Anopheles coluzzii* are all known as major disease vectors. Some of these species can transmit the West Nile, the yellow fever, the dengue fever, and(or) the Rift Valley fever viruses, while others are important vectors for the parasitic roundworm *Wuchereria bancrofti*. **Significance:** Besides providing essential information to set up vector prevention and(or) control measures at deployment sites, the present results also support the importance of treating army equipment appropriately when returning to Belgium in order to avoid unintentional introductions of disease vectors.

A DNA-based approach to validate the identification of exotic mosquito species in Belgium

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Background: Due to international trade, climate and ecological changes, and tourism, mosquito (Culicidae) species are transported, dispersed, or introduced and eventually become established into new territories. Since 2017, a nationwide 3-year project started in Belgium (MEMO: Monitoring of Exotic Mosquitoes), which is funded by the federal government, and which aims at detecting and monitoring the occurrence of exotic mosquito species (EMS) in the country. Activities started with mosquito sampling in 23 points of entry (PoE's), using adult trapping, as well as egg and larval sampling. **Results:** Collected EMS are barcoded to verify their morphology-based species identifications. Also, 5% of the yearly mosquito specimen collection is verified using DNA-barcoding technology (about 1000 specimens), as a quality control measure of the morphology-based species identification. In 2017, 15 native species belonging to five genera were identified and confirmed by DNA data. Additionally, all intercepted EMS could be distinguished from the native Culicidae. Presently, four EMS were collected once or multiple times at one or multiple PoE's: *Aedes koreicus*, *Aedes japonicus*, *Aedes albopictus*, and *Anopheles pharoensis*. In 2018, *Aedes albopictus* was intercepted at five PoE's, three of which for the first time. Also, a new species for Belgium, *Culiseta longiareolata*, was recorded during this nation-wide monitoring project. **Significance:** EMS were found to enter Belgium effectively and repetitively through different introduction pathways: via lucky bamboo and used tyre transport, ground traffic, but also possibly by natural dispersal. In this perspective, MEMO will contribute to a better understanding of the introduction process of the different exotic species by providing information on their status (introduction, establishment, or spread), which is essential to guide EMS surveillance and their control. Also, the DNA-based approach to validate the morphological identifications is essential to ensure the quality of the identifications and to identify stadia which cannot be identified based on morphological characteristics.

Message in a bottle — biodiversity surveys using Malaise traps

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Background: Terrestrial ecosystems are under increasing pressure with consequences such as severe habitat degradation, overexploitation, altered food web dynamics, and shifts in community composition, all leading to a serious decline of diversity. A fundamental tool for providing data to support management of the environment is biomonitoring. However, such assessments take time, come with high cost, and require taxonomic expertise that is not always available. The combination of effective sampling techniques (e.g., Malaise traps) with metabarcoding promises to provide the urgently needed comprehensive approach to monitor communities rather than individual key species. **Results:** Our study assessed spatial variation of species diversity for arthropod communities collected by a network of Malaise traps deployed at a single site in southern Ontario. Our results show only modest levels of species overlap (36%) even for traps that were 30 m apart from each other. About 60% of the total species pool was found only once among an array of 10 traps set in a row with 3 m individual distance. **Significance:** This study represents a first step towards a better understanding of how to efficiently utilize Malaise

traps in large-scale biomonitoring programs. It also highlights the prevalence and impact of transient species in communities collected with intercept traps. The ability to quantify the extent of spatial heterogeneity will allow us to better plan and maintain large-scale biodiversity surveys.

Hidden secrets of biodiversity: DNA barcoding of Hymenoptera suggests a precision in species richness estimates that does not match accuracy

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Background: Quantifying species richness of an area is a common goal for ecologists, but accuracy can be difficult to achieve, and it can be confused with precision. There is underappreciation of the difference between these two goals in biodiversity estimation. Inappropriate sampling and analytical procedures can deliver highly precise, but very inaccurate estimates of true diversity. Understanding this is critical for informing ecologists and other stakeholders on biodiversity hotspots and species at risk. Using hymenopteran species, we show that inappropriate biodiversity sampling regimes can suggest a precision in species richness estimates that does not match accuracy. **Results:** We DNA barcoded (COI) bees (Colletidae, Halictidae, Apidae, Megachilidae) and wasps (Braconidae, Gasteruptiidae) from Australia-wide localities from approximately 1200 specimens, with many of the molecular operational taxonomic units (MOTUs) represented by single specimens. Diversity accumulation curves reveal that species richness of bees and wasps is still extremely under sampled, and estimates of species diversity in Australia are currently difficult to determine, and many more species are yet to be discovered. We then contrasted these results for the Australian Hymenoptera with diversity accumulation curves of bee species richness estimates from 1006 barcoded specimens across three sampling efforts from Fiji. Our results reveal marked differences between highland and lowland species richness and diversity, and this differed markedly with sampling effort, with highland regions harbouring over double the total richness of the lowland. **Significance:** Our initial Fiji-wide samples of bees involving very large sample sizes spread across a large number of islands indicated a low level of species diversity with relatively high precision. However, more regionally targeted sampling that incorporated prior knowledge about species-specific ecologies indicated that this apparent precision was incorrect. Future studies need to be very clear about how biodiversity sampling protocols can produce apparently precise biodiversity estimates and should provide explicit caveats for how their results can be interpreted.

The nestling diet of high Arctic snow buntings (*Plectrophenax nivalis*) assessed by DNA metabarcoding

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Background: The warming climate of the Arctic has been shown to affect different species and populations in diverse ways. Migratory birds reproducing in the Arctic might on one hand benefit from an extension of the summer growth season, but on the other hand could also suffer from mistimings between the timing of reproduction and the peak abundance of food items. The northernmost breeding passerine bird, the snow bunting (*Plectrophenax nivalis*), has shown an advance towards an earlier onset of breeding and a simultaneous reduction of reproductive success during the last two decades in a monitored population on Svalbard. A potential mistiming between the peak of arthropod prey availability and the main nestling period has been proposed, but the actual nestling diet of Svalbard snow buntings has never been assessed. With the help of next-generation sequencing of faecal samples, this study identifies the nestling diet of snow buntings for the first time on Svalbard. The results are linked to the variation in arthropod abundance over the breeding season, detected by pitfall trap sampling. **Results:** The identification of arthropod taxa in the pitfall traps showed dominance of Araneae and Chironomidae in the early season, while later Muscidae constituted the largest fraction. The faeces samples are currently analysed with the help of next-generation sequencing, and the newest results will be presented at the conference. It is expected that the nestling diet will be primarily composed of the same taxa found by the pitfall trap sampling, but might show different compositions indicating preference towards certain taxa. **Significance:** This study identifies for the first time the diet of snow bunting nestlings with metabarcoding methods. The practical use of reference databases like BOLD for studies of diet in remote areas is shown.

Dating West Antarctic ice sheet collapse using molecular sequence data

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West Antarctica has been identified as one of the fastest-warming places on the planet. Large parts of the West Antarctic Ice Sheet (WAIS) are predicted to melt as a result of climate change. Reducing uncertainties over the rate of that melt has been identified as a key research priority in the 5th IPCC Assessment Report. It is well understood from geological reconstructions that there are times in the past when average temperatures were only ~2–3 °C warmer than today, but global sea levels may have been up to 20 m higher. Determining which of these times may have been accompanied by a widespread collapse of the WAIS is needed to provide critical insights into the potential rate and magnitude of sea-level rise over the coming decades and centuries. It is unknown whether there was a collapse of the WAIS during the Last Interglacial, 125 000 years ago—the last time Earth was +1 °C warmer than the pre-industrial period. A recent ice sheet model implies Antarctica may have contributed up to 5 m of the 6–9 m of global sea-level rise known from geological evidence. Confirming this is particularly important for constraining future sea-level projections. The complete collapse of the WAIS would lead to the existence of trans-west Antarctic seaways linking the present-day Ross, Weddell, and Amundsen Seas. Such seaways would allow marine animal migration across newly opened straits, and a genetic signature of that historical connectivity will persist in the genomes of benthic animals present in Antarctica today. I will describe how we are using this information to distinguish between hypotheses to determine when the WAIS last collapsed and how barcoding plays a key role in this work.

Taxonomy at NCBI and GenBank, with a focus on processes and policies pertinent to barcoding projects

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NCBI taxonomy database contains names and classification information of about 440K formally published eukaryote species and subspecies (which have molecular data submitted to NCBI databases). If counting informal species and subspecies names, of which many are submitted for barcoding projects, the number is over 1.2 million. Updates (new taxa and combinations, phylogeny and classification revisions, type and reference information, etc.) to the database are made on a daily basis. A brief introduction is given for the taxonomy data-

base, the group who is responsible for maintaining and developing it, and their operations. Processes and policies for submissions and updates are explained with a focus on how they are related to taxonomists and barcoding projects. The NCBI taxonomy browser, BioCollection, MOLE-BLAST, and other tools will be demonstrated with examples (e.g., how to establish connections between sequence data and museum collections). Worldwide DNA barcoding projects have been deeply involved in the work of NCBI taxonomy. In recent years, we have received related submissions and requests for updates for huge numbers of records. For example, there were organism name updates for approximately 700K GenBank accessions from BOLD in 2017. Accordingly, the taxonomy group has frequently re-examined current treatments and policies in order to record and present information in a meaningful and sustainable way. We also look forward to developing and improving channels for communications and pipelines for updates from other institutes, facilities, and databases. Through this presentation I also hope to answer questions and receive feedback and suggestions from colleagues and users on how NCBI can provide better service to the research community.

The spatio-temporal distribution of insects across Denmark assessed by citizen science and DNA

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Studies show drastic declines in insect biodiversity in temperate as well as tropical climates over the last decades. Broad-scale studies and monitoring schemes on insects are rare and usually focused on specific groups, e.g., pollinators or pests. However, insects maintain several important functional roles in terrestrial ecosystems; hence, knowledge of the distribution of insect biodiversity is needed to understand the drivers of insect decline and potential consequences for ecosystem food webs. Citizen Science enables large-scale sampling, and metabarcoding provides the means for processing large sample quantities. This study will examine the spatio-temporal distribution and diversity of flying insects in five specific land-use types in Denmark by drawing upon both citizen science and metabarcoding. Sampling of flying insects is performed by more than 300 volunteers with large nets mounted on the rooftop of their car during June 2018 and 2019 along predefined routes under specific weather conditions. Over 1200 bulk insect samples are size sorted in two size fractions, and the largest size fraction is assigned morphological taxon IDs. Sample dry weight is measured prior to nondestructive DNA extraction and tagged PCR amplification with three universal insect primer pairs. Pooled PCR products are sequenced with Next-Generation Sequencing. The results will be compared to land-use, bird fauna, and biodiversity data to generate food networks and thus quantify the significance of land use for Danish biodiversity. Furthermore, this study may have an impact to influence nature management policies and has the potential to be a cornerstone for broad-scale monitoring of insect biodiversity.

Evaluating airborne pollen biomonitoring with metabarcoding in Germany

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Background: Long-term monitoring of airborne pollen provides important information for human health in addition to environmental information regarding flowering phenology, species composition, and invasive species range detection. Despite this, long-term publicly funded airborne pollen monitoring occurs only in a few countries and primarily in the northern hemisphere. Pollen monitoring is traditionally done with time-intensive morphological identifications that require extensive training. DNA metabarcoding has the potential to provide faster, less-expensive identifications with improved taxonomic resolution and without experimenter bias. However, in order to implement DNA metabarcoding in large-scale biomonitoring programs, several limitations inherent in plant barcoding, pollen genetics, and airborne PCR inhibitors must be overcome or accounted for. **Results:** This study examines metabarcoding of airborne pollen throughout an entire year (2016) in three sites in northern Germany (urban Bremen and Berlin, and a rural area 25 km west of Bremen) and directly compares the results with morphological identification. We evaluated the performance of four different aerobiological collection methods that vary in capacity, adhesives, and liquid handling. DNA extraction method and PCR were optimized to overcome by-catch PCR inhibitors, and three common plant barcodes were evaluated (ITS1, ITS2, rbcL) for species identification. **Significance:** In order to incorporate DNA metabarcoding into large-scale pollen monitoring programs, extensive method standardization and testing of accuracy must be performed. Nevertheless, in times of diminishing taxonomic expertise, automated high-throughput pollen identification technologies are in high demand. This study provides an evaluation of current methods used for pollen metabarcoding and where improvements could be made in order for resulting identifications to be considered reliable.

Thriving through the Ice Age: European proglacial refugia and genetic diversity of a widespread freshwater isopod

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Background: Common waterlouse, *Asellus aquaticus*, is one of the most widespread and common macrocrustaceans inhabiting a variety of fresh waters all over Europe, including the vast post-glacial areas freed from the Pleistocene ice sheet only at the beginning of the Holocene. Given the results of previous studies upon the phylogeography of the species, showing relatively high genetic diversity even in the northern areas of Europe, it may be expected that some populations of the species did not migrate to this region from southern Europe but survived in local periglacial refugia. **Results:** Our dataset included 740 COI and 560 ITS2 sequences from 178 localities all over extra-Mediterranean Europe. We identified Pontic Basin as a major periglacial refugium of *A. aquaticus* and a high migration rate between this region and the Central European Plains. No decrease of population size in central and northern Europe was observed during the Last Glacial Maximum. Apparently, the vast system of proglacial lakes forming on the margins of the European ice sheet supported the waterlouse population. Trace fossils from various parts of northern Europe seems to support this scenario. **Significance:** The story of *A. aquaticus* turned out to be not much of what we have expected taking lessons from other aquatic taxa. Moreover, it makes us to stop perceiving the proglacial environment as an ice desert, showing that perhaps it was filled with variety of life forms. Eventually, we should reconsider the small crustacean, waterlouse, as a persistent traveller around Europe and, feasibly, other continents, which requires further attention and investigation of dispersal abilities and ecological requirements of particular genetically divergent populations.

DNA barcoding in jumping bristletails (Archaeognatha): the Austrian species of *Lepismachilis* and *Dilta*

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Background: Pigmentation patterns of the compound eyes are the most important characters to delimit Central European species of *Lepismachilis*. Unfortunately, the pigmentation is fading in specimens stored in ethanol. Therefore, in the only voucher of *L. notata* from Austria, species affiliation cannot be confirmed anymore. Thus, the most recent checklist mentions only *L. y-signata* and *L. rozsypali* to reliably occur in Austria. All Austrian specimens of *Dilta* up till now were tentatively affiliated to *D. hibernica*. Species affiliation mainly relies on biogeographic reasoning. A final confirmation is impossible, since only parthenogenetic populations are present in Austria, in a genus where only males can be determined to species level. **Results:** Our study re-established *L. notata* as part of the Austrian fauna. The pigmentation pattern thought to be specific for *L. y-signata*, however, was found in three well-separated clusters, tentatively representing three distinct species. Our study likewise found two distinct clusters in *Dilta*. One cluster may represent a species known from the western Alpine region. **Significance:** The number of tentative species of *Lepismachilis* known from Austria increased to five. The dark mark in the form of a Y most probably represents the plesiomorphic character state in compound eye pigmentation. DNA barcoding thus may help to polarize evolutionary characters. Additionally, it potentially allows for species affiliation of parthenogenetic populations in genera where only the male sex can be determined. Unfortunately, DNA barcodes from male specimens are still missing from BOLD in *Dilta hibernica*.

The importance of experimental design in metabarcoding studies

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A DNA metabarcoding experiment seems easy to implement according to the simplicity of its different steps consisting of (*i*) sampling and extracting environmental DNA, (*ii*) amplifying a metabarcode, (*iii*) sequencing on a next-generation sequencer, and (*iv*) sequence analysis based on published bioinformatic pipelines. Unfortunately, this is not the case, and metabarcoding suffers from many difficulties due to several categories of experimental artefacts. First, the sampling protocol might not collect the expected biodiversity. Second, the amplification step might introduce a strong bias among the different target taxa. Third, the "tag jump" problem can lead to false positives. This is particularly true for the most common taxa. Fourth, the "index jump" problem can also erroneously show the presence of a taxon in a sample if several libraries using the same primers are loaded on the same sequencing lane. Finally, relaxed filtering thresholds during the bioinformatic analysis might also generate false presence. Knowing all these potential problems, it is possible to design an experimental protocol that will limit their impact and secure the final results. To cope with sampling problems and to know the variance of the results for each sample, it is necessary to include several biological replicates. To limit the impact of the stochastic aspect of PCR, several technical replicates must be considered. The setup of many positive and negative controls allows to adjust the filtering steps, and to deal with several potential problems such as sporadic contaminations. We will also discuss additional technical considerations that improve the overall results of a metabarcoding experiment.

DNA barcoding of spiders from agricultural fields of Layyah, Pakistan

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Background: Species identifications on the basis of morphological characters is not only time consuming but also problematic because of several reasons. Researchers now are using DNA barcoding for species identification. It has accelerated the pace of species discovery. In the present study, DNA barcoding was used to assess the percentage accuracy of morphology-based identification of spiders collected from agriculture fields of district Layyah, Punjab, Pakistan. **Results:** A total of 872 spiders was captured during a sampling period of three months, from June 2017 to August 2017. All the field-collected spiders were brought to a molecular laboratory at GC University Lahore, preserved in 95% ethanol and stored at -20°C until the DNA extraction. Spiders were evaluated morphologically on the basis of different identification keys and catalogues. Morphological identification revealed the presence of 12 families, 29 genera, and 49 species. To evaluate the authenticity of morphological identification, tissue samples of 96 specimens were sent to the Canadian Centre for DNA Barcoding, University of Guelph, Canada. In total, 658 base-pair sequences of COI (cytochrome c oxidase subunit I) of 90 specimens were retrieved successfully, which confirmed the presence of 11 families, 25 genera, and 47 species. On the basis of the molecular results, all the misidentified specimens were then allocated to the correct taxon. Overall, the accuracy of morphology-based identification was 88%. **Significance:** It is concluded from the present study that morphological investigations to identify a spider are satisfactory, but to enhance the accuracy and credibility of results, molecular methods like DNA barcoding are inevitable. Furthermore, to magnify the pace and authenticity of the evaluation of spiders, integrated barcoding—a combination of molecular methods and conventional taxonomy—is compulsory.

DNA barcodes reveal extensive hidden diversity in polychaetes, questioning cosmopolitan distributions and calling for comprehensive taxonomic revision

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Background: The Polychaeta (Annelida), one of the most prominent bioindicator groups and a well-represented class of organisms among the marine benthic invertebrates, seems to be particularly afflicted by the frequent occurrence of cryptic species. Due to their abundance, cryptic species can in no way be neglected if we want to correctly assess species diversity and distribution, understand biogeographic patterns, or to keep track of natural or anthropogenic-induced changes in marine communities. In our study, we have been conducting comprehensive morphological and molecular analyses of the polychaetes, mainly from the order Phyllodocida in the NE Atlantic, to investigate cryptic diversity in this group. Given the publicly available data for comparison and well-known patterns of variation, we used the cytochrome c oxidase subunit I barcode region (COI-5P) to screen for intraspecific genetic divergence in suspected cryptic complexes, based on prior evidence. **Results:** Applying four different molecular operational taxonomic unit (MOTU) clustering algorithms, we detected one additional MOTU present only in the UK within the large *Eumida sanguinea* complex, comprising nine different described morphospecies (18.8% mean K2P distance); five additional MOTUs within the *Eulalia viridis*/*clavigera* complex with 17.3% mean distance, where

one of the them is exclusive to the Madeira island; seven MOTUs within *Trypanosyllis zebra* (22.1% mean distance), where three are from the Mediterranean; and four MOTUs within *Platynereis dumerilii* with 17.9% mean distance, with two lineages endemic to Portugal. **Significance:** With much narrower ranges and vulnerability when compared to cosmopolitan species, these complexes of cryptic species may need extra consideration for conservation practices. We aim to use this class of invertebrates as a model to gain insights into this still poorly understood evolutionary phenomenon, while also improving the DNA barcode reference library for polychaetes, which can be used in conjunction with high-throughput sequencing technologies for biomonitoring programmes or other relevant ecological research.

Dietary niche partitioning in domestic and wild herbivores in southern India using DNA metabarcoding

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Background: Wild herbivores are not only under pressure from habitat degradation and destruction, but also have to compete for resources with each other and with domestic animals. India has the world's second largest livestock population, which is grazing many of India's wildlife reserves. This is a major cause for concern as livestock has competitive advantages over the local wildlife. Therefore it is important for conservation purposes to understand the dietary niche partitioning between these herbivores. DNA metabarcoding previously provided information about the dietary composition for a range of different animals and has been applied here to reconstruct the diet of herbivores present in the Malai Mahadeswa wildlife sanctuary, southern India. **Results:** More than 100 faecal samples are collected in the Malai Mahadeswa wildlife sanctuary from a range of different herbivores, including elephants, cattle, goats, muntjacs, buffalos, wild boars, and macaques. Analysis of the herbivore DNA present in these samples allows for robust identification of the species, while the plant DNA provides insight into the dietary composition. For this purpose, DNA metabarcoding primers targeting the herbivore taxa of interest, and the universal plant primer pair trnL-g/trnL-h are used to amplify DNA from the collected samples. **Significance:** The impact of livestock on wild herbivores is a global conservation concern. With increasing pressures on local wildlife from a range of different factors, DNA metabarcoding of faecal samples is a non-invasive method providing a wide variety of information that could prove vital for the preservation of biodiversity in wildlife sanctuaries.

Monitoring fish communities in Alpine rivers via eDNA

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Background: Assessing the composition of fish communities in river systems is crucial for defining the ecological status of these habitats and, if necessary, establishing effective plans for management and stocking. Environmental DNA (eDNA) has the potential to revolutionize fish monitoring in rivers as it enables the analysis of multiple samples from different locations within river networks. This allows us to easily pinpoint upstream distribution limits for individual species and longitudinal changes. Although Alpine rivers are characterized by a rather simple fish community structure, low population densities coupled with high discharge fluctuations represent a challenge for broad-scale application of eDNA-based fish monitoring. **Results:** We assessed the suitability of 12S metabarcoding to characterize fish communities in Alpine rivers of different size and zonation (epirhithral to epipotamal), including samples taken at or above known upstream distribution limits and along ~150 km of the River Inn. To test the sensitivity of this approach, the results were compared to species-specific diagnostic PCR and electrofishing data. With respect to frequently occurring species in the Inn, the metabarcoding approach was found to be highly suitable for assessing longitudinal changes. However, when compared to diagnostic PCR and electrofishing, reliable detection via metabarcoding was not always possible close to respective upstream distribution limits and for rare species in general. For samples taken at the same site, the variation in read numbers was not congruent with fluctuations of eDNA signal strength derived from diagnostic PCR coupled with capillary electrophoresis. **Significance:** The combination of the two molecular approaches highlights the potential of eDNA for future large-scale fish monitoring in Alpine rivers. 12S metabarcoding is appropriate to assess longitudinal changes in fish communities at a large scale, whereas diagnostic PCR outperforms both metabarcoding and electrofishing in locating upstream distribution limits.

Metabarcoding reveals chironomid biodiversity loss and species turnover due to mosquito control actions in temporary wetlands of the Upper Rhine Valley

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Background: The Upper Rhine Valley, one out of 30 Hotspots of Biodiversity in Germany, has been treated with the biocide *Bacillus thuringiensis* var. *israelensis* (*Bti*) for decades as an environmentally friendly alternative for mosquito control. Previous studies discovered *Bti* nontarget effects with severe chironomid abundance reductions. In this study, we investigated the impact of *Bti* on the nontarget family Chironomidae and addressed the community composition by use of state-of-the-art community metabarcoding. **Results:** Chironomid emergence data were collected in three mosquito-control relevant wetland types. For all three sites, the chironomid community composition, based on operational taxonomic units (OTUs), was altered to varying degrees in the *Bti*-treated samples versus control samples, ranging from a significant 63% OTU reduction to an OTU replacement. Although we assumed that predatory chironomids are less prone to *Bti* than filter-feeding species, a comparable percentage of predatory and filter-feeding taxa (63% and 65%) was reduced in the *Bti* samples, suggesting that the feeding strategy is not the main driver for *Bti* sensitivity in chironomids. Finally, our data revealed a chironomid community recovery due to species recolonization after a few years of *Bti* intermittence. **Significance:** Our study demonstrates that the application of the biocide *Bti* can result in chironomid biodiversity loss and species turnover. Considering the very diverse chironomid communities in terms of species composition and age structures at different wetland types, the *Bti* effect can be highly variable, depending on the time and mode of the *Bti* application. The *Bti*-induced quantitative and qualitative alterations of chironomid communities might have severe consequences for the wetland ecosystems. Considering the currently discussed insect reductions, we recommend a rethinking of the usage of the biocide *Bti*, especially in nature protection reserves to enhance ecological resilience and to prevent boosting the current biodiversity loss.

Effect of bioinformatic pipeline on bioassessment index performance

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Background: DNA metabarcoding provides a rapid, accurate, and scalable alternative to traditional morpho-taxonomic approaches for generating robust taxonomic data for bioassessment applications. However, protocols for the generation and analysis of DNA data, including bioinformatic processing, are not yet standardized, and individual analytical pipeline choices can bias resulting species composition data and therefore site bioassessment scores. To better understand the influence of bioinformatic processing steps on biological index scores, we performed metabarcode sequencing (16S and 18S rRNA gene) on 80 stream algae samples collected from the Santa Margarita River in southern California. We processed the sequence data using two separate bioinformatic pipelines (MOTHUR, QIIME) for quality screening and operational taxonomic unit (OTU) clustering (at 95%, 97%, 100% levels). Additionally, we compared OTU and individual sequence variant (ISU) abundances to evaluate the option of eliminating multiple bioinformatic processing steps and their potential biases. For all bioinformatic output, we assigned taxonomic identity to OTUs or ISUs and calculated California algal index scores (ASCI) for diatoms and soft-algae. **Results:** Across the seven bioinformatic treatments tested, we found a high degree of similarity in species composition and relative abundances of dominant taxa. Algal index scores were highly consistent across bioinformatic treatments, including those calculated from the ISU sequence data. Notably, ISU index scores were within the margin of variability observed across field replicate samples, demonstrating that ISU sequences generated with minimal bioinformatic processing offer an attractive alternative to lengthier bioinformatic pipelines. **Significance:** Our results show that minimally processed ISU sequences yield comparable index scores to highly curated OTU-based data. The elimination of unnecessary bioinformatic processing not only has the potential to help democratize DNA-based approaches, but also to improve consistency, reliability, and accuracy of DNA-based biological index scores, therefore paving the way for the broad-scale adoption of molecular approaches for bioassessment programs.

Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods

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Background: Terrestrial arthropods comprise the most species-rich communities on Earth, and grassland flowers provide resources for hundreds of thousands of arthropod species. Diverse grassland ecosystems worldwide are threatened by various types of environmental change, which has led to a decline in arthropod diversity. For instance, pollinators like bees and butterflies represent an important ecological group that has undergone severe decline in Europe, indicating a dramatic loss of grassland biodiversity. At the same time, monitoring grassland arthropod diversity is time-consuming and strictly dependent on declining taxonomic expertise. Environmental DNA (eDNA) metabarcoding of complex samples has demonstrated that information on species compositions can be efficiently and noninvasively obtained. Here, we test the potential of wild flowers as a novel source of arthropod eDNA. We performed eDNA metabarcoding of flowers from several different plant species using generic arthropod primers. **Results:** Our results show that terrestrial arthropod species leave traces of DNA on the flowers that they interact with. We obtained eDNA from at least 135 arthropod species in 67 families and 14 orders, together representing diverse ecological groups including pollinators, parasitoids, gall inducers, predators, and phytophagous species, and arthropod communities clustered together according to plant species. **Significance:** This novel source of eDNA represents a vast potential for addressing fundamental research questions in ecology, obtaining data on cryptic and unknown species of plant-associated arthropods, as well as applied research on pest management or conservation of endangered species such as wild pollinators. However, a range of further experiments to obtain better insights into the nature of arthropod eDNA on flowers would be highly beneficial for future applications. Finally, our results also indicate that eDNA might be much more abundant in the environment than one would immediately imagine.

New insights on the evolution of the chasmophyte *Silene*: a case study of section Italicae (Caryophyllaceae)

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Background: *Silene* section Italicae (Caryophyllaceae) comprises a number of Mediterranean species showing differing life forms, a wide range of habitats, and various shapes in their distribution. The section, with about 30 species being described, has been taxonomically controversial due to the ambiguous species boundaries. Such obscure species limits can be the result of recent divergence events, where time has been too short to allow for morphological differentiation. On the other hand, morphological similarities can result from adaptation to similar environmental conditions, or specific conditions can lead to morphological variations while species show no significant genetic differentiation. In this study, using DNA sequence data from sections Italicae, Paradoxae, Giganteae, Siphonomorpha s.str., of genus *Silene*, we attempt to understand the species delimitation and evolution of the chasmophyte species of the section Italicae under the Bayesian framework. **Results:** Our analyses revealed largely concordant results with the previous studies of the group, considering the contents and the relationships of the section. The relationships among the four sections remain unclear. However, chasmophyte species of the group were observed as distributed all over the species tree, indicating convergent morphological evolution driven by parallel environmental conditions. **Significance:** This study suggests that the switch between open rocky and chasmophytic habitats occurred several times within the corresponding sections of genus *Silene*.

Tardigrade diversity assessment using morphology, DNA barcoding, and multi-marker metabarcoding

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Background: Water bears (Tardigrada) are microscopic animals found in all parts of the world—from deep marine sediments to alpine environments. These invertebrates are renowned for their ability to enter an anhydrobiotic state that allow them to endure extreme conditions for prolonged periods. Despite their celebrity status, the Norwegian tardigrade diversity has received little attention. In this study, we use morphology and molecular tools to identify the tardigrade biodiversity in an oak-linden forest in southern Norway, and we investigate if metabarcoding of environmental DNA (eDNA) samples accurately reflects the recorded diversity. **Results:** By investigating samples of litter, lichen, and moss (five samples for each substrate), we found on average 203 individuals and 11 morpho-species per sample, totalling 37 different species among 3040 specimens. DNA barcodes were retrieved using COI universal primers from 1–15 individuals of as many as possible of these species, depending on their abundance and our a priori knowledge of possible cryptic diversity of each species. eDNA of the same moss, lichen, and litter samples (15 in total) was extracted and metabarcoded using two modified primer pairs for COI

and one universal primer pair for 18S. We report on the observed similarities and differences between individual identification and metabarcoding of eDNA from the same samples. **Significance:** As other meiofauna, tardigrades are neglected in most ecological and environmental assessments. They are, however, an important component of terrestrial microscopic biodiversity, and we need to expand our knowledge of their ecology and evolution. Metabarcoding of environmental samples may provide an effective way of incorporating tardigrades and other microscopic multicellular life in environmental assessments. This study provides a step in this process and indicates if metabarcoding of forest eDNA gives a representative picture of the recorded tardigrade diversity.

From theory to practice: the development and validation of an eDNA assay as a commercial method for the detection of white-clawed crayfish

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Background: The white-clawed crayfish (*Austropotamobius pallipes*) has suffered from a large decline in numbers across Europe since the introduction of several non-native and invasive crayfish species, resulting in its status being reclassified as endangered. Currently within the UK, there are limited efforts to detect existing or potentially present populations of *A. pallipes* due to sporadic locations and isolation of sites, the cost of sampling a large area, and the amount of time which is required to do such survey work. The application of environmental DNA (eDNA)-based species detection could vastly improve the efforts made to detect, monitor, and in turn conserve populations of the species. However, despite the apparent promise of eDNA, there are still several variables and limiting factors that must be addressed before such a technique can be employed on a large scale by future end-users. **Results:** Working alongside stakeholders, commercial organisations, ecologists, and end-user groups in the UK, we have validated eDNA sampling for *A. pallipes* for use as a commercially available tool, through the careful assessment of the variables and limitations known to affect eDNA. We report differing detection probabilities when using different sample collection methodologies, including filtration and precipitation, indicating the importance methodological design. The impact that environmental variables and seasonal conditions have on detection success is also reported, highlighting eDNA degradation rates and the effect that seasonal variations ultimately have on the ability to detect and monitor populations of *A. pallipes*, leading to a number of recommendations on sampling collection approach and season. **Significance:** By identifying, testing, and feeding back the impact that many of the significant variables have into the design of the assay, we improve accuracy and reliability of the approach, therefore positioning eDNA testing as a viable additional survey method for the presence/absence of *A. pallipes* on a commercial scale.

How to make the field guide "DNA of forests"

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As the cost of DNA sequencing has decreased drastically for the last decade, DNA barcoding has spread widely as a method that enables nonprofessionals to identify species. However, opportunities to learn the methods are still scarce in Japan. While the results of DNA barcoding are archived in online databases, the visualization still has room for improvements. Especially, visualization of the omics data with local geography and landscape is needed to understand the relationship to the environment around us. With this in mind, we developed a public workshop called How to make the Field Guide "DNA of Forests" in 2016. At the workshop, the participants collect living samples in the forests and observe them through a microscope. Participants then prepare DNA samples using extraction kits, run PCR, and sequence samples by using sequencing services or portable sequencing devices. They then use bioinformatics, including BLAST search, to identify the species collected. The workshop has taken place three times so far and has involved a wide range of citizens, such as elementary school students, high school teachers, museum researchers, designers, engineers, active seniors, etc. We have also constructed an online archive presenting the results of the participants' observations and DNA analysis. Here, you can have a 360-degree panoramic view of the surrounding forest and draw out information collected by the workshop participants. The project aims to provide and cultivate multiple perspectives by collecting such latent information and compiling them into one field guide. https://special.ycam.jp/dna-of-forests/en/#/.

Contractual solutions

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A contract is the primary legal tool for implementing the benefit sharing obligations. Often, research projects starts out with low commercial expectation. The situation where academic research finds a lead with commercial prospects is not an atypical one. Therefore, the situation where so-called noncommercial research turns out to become commercial is a normal situation. This implies that a contract for access and benefit sharing (ABS) needs to deal with these typical changes in purpose and used from academic to commercial applications. This presentation will explore how contract clauses can capture this kind of change. In ABS, the topic of digital sequence information (DSI)/data and synthetic use of biological material are core questions in the ongoing political discussions. Here, it will be explored standard clauses to include DSI and utilisations of the biological material in a synthetic manner. How can contracts contribute to realising the commercial value of academic research based on biological resources?

Towards a genomic barcode for resolving the recent species radiation of eyebrights (*Euphrasia*, Orobanchaceae)

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Background: Scaling up from standard DNA barcodes to complete plastid genomes may improve species discrimination in recent species radiations. We test the efficacy of plastid genomes for telling species apart in a postglacial species radiation of eyebrights (*Euphrasia*, Orobanchaceae). We focus on understanding the evolutionary processes that may obscure the genetic signal of species differences, including nuclear plastid DNAs (NUPTs) and heteroplasmy. **Results:** Plastid genomes provide sufficient sequence characters to identify numerous unique haplotypes, whereas extensive haplotype sharing is seen for any single plastid DNA barcoding locus. However, phylogenetic analysis of plastid haplotypes shows an unexpected and likely implausible pattern of complex geographic structure. Genomic characterisation of sequence reads suggests heteroplasmy, as well as numerous events of plastid integration in the nuclear genome, are potential sources that affect the assembly and interpretation of plastid sequences. **Significance:** Complete organelle genome sequences have been suggested as a viable genomic barcode, but these may be subject to evolutionary processes that make them a poor proxy for nuclear genomic differentiation. These processes may obscure the genetic signature of species differences in recent species radiations. In the most taxonomically complex groups such as *Euphrasia*, nuclear genomic information analysed in a population genetic framework may prove the only definitive way to reliably tell species apart.

Miocene dispersal and diversification of climbing palms ('Rattans', tribe: Calameae, Arecaceae) in Indian subcontinent

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Background: The origins of patterns of high plant diversity in Asian tropical forest communities have long been a subject of controversy. One hypothesis suggests that plants of Gondwanan origin may have dispersed eastward from the Deccan plate (South Asian region) and diversified in the Far Eastern (Malaysian) region of the Eurasian tectonic plate. The alternative hypothesis suggests that flora of the Far Eastern region may have dispersed westward, contributing to high plant diversity in South Asia. **Results:** In order to evaluate these conflicting hypotheses that centralise the Indian subcontinent, we performed phytogeographic, phylogenetic, and molecular dating analyses of the tribe Calameae of family Arecaceae. The analyses of chloroplast and nuclear DNA sequences in combination with morphological data revealed an evidence for Miocene diversification and multiple dispersals of the tribe Calameae across Indian Ocean. The biogeographic analyses using phylowood and S-DIVA show the spatial and temporal diversification of climbing palms in India. **Significance:** The current study sheds light on the systematic relationships within the tribe Calameae and the biogeographic history of this ecologically interesting group. The diversification of this taxon, predominantly in the Miocene, unravels the in-situ diversification in the Western Ghats of India, and islands of the Andaman and Nicobar archipelago also evidently played a role as stepping stones in the trans-oceanic dispersal of Indian calamoids.

Two-tiered authentication of Nilavembu Kudineer, an herbal decoction treating dengue and chikungunya using DNA barcodes and HPTLC fingerprints

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Background: Nilavembu Kudineer also called Vishasura Kudineer (Deadly-fever water) in Siddha medicine (derived from Tamil; Visha meaning poison; sura meaning fever; Kudineer meaning drinking water) refers to the formulation of eight herbals and has been mainly used for treating viral fever, especially against dengue and chikungunya. The formulation largely contains 80% *Andrographis paniculata*, 10% *Hypertelis cerviana*, and 10% of six other herbs. These herbs are collected directly from the wild, but it is doubtful if wild populations are large enough to meet the high demand. This study aimed to authenticate the content of the powder-form product using DNA barcodes and HPTLC fingerprints. **Results:** A phylogenetic analysis using MrBayes based on rbcL DNA barcodes showed topological clustering and phylogenetic differentiation between authentic and mixed species in the powder of Nilvembu Kudineer. It revealed the presence of at least 20 different species, including the eight reference species of this herbal decoction. The majority of the raw drug samples were *A. paniculata* (86%), *H. umbellata* (8%), *Vetiveria zizanioides* (2.2%), and *Piper nigrum* (2%). The remaining raw drug samples were *H. cerviana* (1.2%), *Trichosanthes cucumerina* (0.5%), and other herbs (1.1%; 14 spp.). **Significance:** The rbcL marker effectively discriminated the different species found in Nilvembu Kudineer. The observed fraudulence was due to either an indeliberate mixture of taxonomically related species or the deliberate mixture of unrelated to authentic species. This deliberate mixture was due to an emergency need to meet the domestic demand to treat dengue in India. The admixture is most unlikely a fraudulent substitution, but the adulteration was perhaps due to their known medicinal properties against fever. However, there is no pharmacological information to justify this substitution to be effective and safe for medicinal use so far.

Identifying freshwater fish larvae through DNA barcoding a tropical oligotrophic lake from southeast Mexico

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Background: Due to insufficient morphological diagnostic characters and the size of larval fish, it is often difficult to key them to species or even genus level. They are therefore often misidentified, especially in studies on freshwater tropical fish. Here, we present the first study to identify the fish larvae from Bacalar Lagoon, Mexico, using traditional methods and the mitochondrial gene COI. The samplings were carried out during 2015 in three seasons: dry (April, June), rainy (July, August), and northern (December), using new methodologies recently proposed for freshwater zooplankton: light traps. **Results:** A total of 2369 fish larva were collected, of which 112 were selected for their morphological identification. Sixteen morphotypes were identified. A sample of tissue was extracted from them and used to produce DNA barcodes. Only three larvae were identified to species level using just morphology (*Bathygobius soporator*); the remaining specimens were identifying to genus (*Gobiosoma* sp., *Ctenogobius* sp.), to family (Engraulidae and Gobiidae), and to order (Perciformes). From the 101 positives, we found a total of six species (*Dorosoma petenense*, *Cyprinodon artifrons*, *Bathygobius soporator*, *Lophogobius cyprinoides*, *Gobiosoma yucatanum*, and *Ctenogobius fasciatus*) and one family (Engraulidae). *Gobiosoma yucatanum* is a brackish species found for the first time in Bacalar. **Significance:** These results are a first approximation to understand the breeding systems of the fish in this lake. They will contribute to the management and conservation of this unique freshwater system, which has a rich endemic fauna and flora and the biggest stromatolites in the world.

DNA barcoding and identification of intermediate slug hosts in the framework of an epidemiological survey in Germany

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Background: Recently, metastrongyloid lungworms that infect canids and felids have gained special attention due to their increasing prevalence in domestic and wildlife populations. There is evidence that infections of *Angiostrongylus vasorum*, the canid lungworm that can cause severe bleeding disorders or breathing distress, are spreading beyond endemic areas by means of terrestrial gastropods. A total of 2701 slugs was collected throughout one year in four areas (two in Hesse and two in Rhineland - Palatinate) that were previously shown as hyperendemic for

A. vasorum infections in foxes. Slugs were identified based on morphological characteristics, yet 16 identifications required validation by DNA barcoding through the Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo). **Results:** DNA sequences were generated for the standard mitochondrial COI barcode, as well as for 16S ribosomal RNA. Using BLAST, the sequences were compared to the reference sequences available in GenBank and BOLD to identify the slugs. They were positively identified by both COI and 16S as belonging to either *Arion vulgaris* (syn. *Arion lusitanicus* Auct.) ($n = 14$) or *Deroceras reticulatum* ($n = 2$). **Significance:** This epidemiological study suggests that, in the surveyed areas, the prevalence of *A. vasorum* is significantly higher in *Arion vulgaris* than in *D. reticulatum*. The current data also imply that in these areas dogs are at permanent risk for *A. vasorum* infections throughout the year. Currently, BopCo is collaborating with the Aristotle University of Thessaloniki to apply DNA barcoding for the identification of Greek snails and slugs in another epidemiological study.

Study of adulteration in the market samples of saffron from fourteen countries using pharmacognosy, HPLC, HPTLC, and DNA barcoding

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Background: Saffron (*Crocus sativus* L.) is a popular spice used in many cuisines. According to ISO, saffron is the World's most expensive spice with a trade value of about 30 €/g. It has a significant role in traditional medicines like Indian System of Medicine (Ayurveda, Siddha, and Unani), Chinese and Persian Traditional Medicine, etc. High cost, restricted production, and lack of stringent authentication methods make saffron prone to adulteration. **Results:** Authentic saffron collected from Kashmir Valley of India was used as reference material. We collected 101 market samples of saffron from 14 countries and did a comparative study by using pharmacognosy (microscopy and macroscopy), HPLC, HPTLC, and DNA barcoding for authentication. The pharmacognosy method identified that 39 samples were nonauthentic. HPLC analysis was done based on safranal-specific peak and a saffron-specific fingerprint. HPLC revealed that 38 of the 39 samples were adulterated. One sample was missed because it was a mixture of authentic saffron and adulterant. HPTLC identified one extra sample as not-authentic as it was adulterated with a dye. DNA barcoding using the rbcL marker revealed 44 samples as being not-authentic. In this, five samples that were classified as authentic by other methods were found to be a mixture of more than one botanical species, other than saffron. Botanical admixture found in saffron market samples were *Ricinus* sp., *Euphorbia* sp., *Jatropha* sp., *Calotropis* sp., *Momordica* sp., etc. **Significance:** This study found that 44% of the saffron market samples were not authentic. Nonauthentic samples contained both botanical adulterants as well as dyes and synthetic safranal as chemical adulterants. Some of the botanical adulterants may have toxic or adverse effects on human health. In general, fewer nonauthentic samples were found among the samples with a higher market price. These results indicate the necessity for authentication of saffron and the need for a combinatorial approach.

Cryptic diversity in the cave planthopper *Kinnapotiguara troglobia* (Hemiptera, Fulgoromorpha, Kinnaridae): evidence from DNA barcodes

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Background: Kinnaridae is a small family of planthoppers from the New World, with most of the described species occurring in North and Central Americas. Only recently, some attention has been given to South American taxa, and a few monotypic genera have been described in the last decade, such as the troglobite *Kinnapotiguara troglobia* from northeastern Brazil. Populations of *K. troglobia* were described inhabiting limestone caves around the Apodi River in Rio Grande do Norte, with an indication of a slight morphological variation. Therefore, we aimed to characterize the genetic diversity within *K. troglobia* through DNA barcoding with COX1 (84 specimens from 11 caves) using three different clustering approaches to check for the distribution of genetic groups: (1) the similarity-based ABGD; (2) traditional phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI); and (3) the multispecies coalescent model in BPP, to evaluate the statistical significance of the obtained groups in the previous approaches. **Results:** The ABGD analysis indicated six well-delimited groups within *K. troglobia*, considering prior intraspecific divergence values ranging between 0.001 and 0.0264, each corresponding to an individual limestone outcrop. The ML and BI trees presented high support values for the six main clades presented, corresponding to the ABGD clusters. Moreover, the BPP analysis strongly supported the division of *K. troglobia* into six species (0.9988), all presenting very high individual posterior probabilities (between 0.9992 and 1). **Significance:** Although resulting from single-locus analyses, our data revealed remarkably high divergences among the major genetic clusters of *K. troglobia*, indicating its separation into six distinct species, as previously suggested considering the variation in the shape of the dorsal process of the parameres. Nevertheless, associating genetic profiles to the morphologic variation, and determining the whole geographic range of Kinnapotiguara in the Apodi mountain range, must be completed before formal taxonomic changes in the group.

Use of diatom DNA metabarcoding for freshwater quality assessment: progress and prospects in the frame of French rivers monitoring networks

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Background: Environmental DNA (eDNA) and metabarcoding approaches has been extensively developed over the past decade as new tools for biodiversity assessment. Among the various applications offered, DNA metabarcoding of benthic diatoms has rapidly shown its potential for freshwater quality assessment. Since then, several optimizations and developments were proposed for the major steps of the metabarcoding workflow: taxonomic resolution of DNA barcodes, efficiency of DNA extraction methods, completion of a barcode reference database, and use of correction factors to obtain taxa quantification equivalent to microscopy. Placed end-to-end, those improvements make the DNA metabarcoding approach a viable approach for quality assessment of rivers in monitoring networks, as effective as the morphological approach (microscopy) routinely applied. **Results:** We set up this bioassessment innovative approach, using the diatom rbcL barcode, at the scale of river monitoring networks in France in the context of the Water Framework Directive (WFD). The DNA-metabarcoding approach proved to be faster than the classical one and economically viable at the scale of large monitoring networks. Molecular-based quality index values were highly correlated to morphological ones and congruent with the river quality status. Correlation between molecular and morphology-based indices were increased by the completion of the reference database and the use of correction factors on metabarcoding data. **Significance:** Our results confirm the potential of DNA metabarcoding for bioassessment, but also raised questions about the need for standardization and harmonization prior to its implementation in biomonitoring campaigns.

Application of eDNA methods for biomonitoring of Alpine lakes and rivers: the Eco-AlpsWater project for innovative ecological assessment

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The EcoALpsWater project aims to improve the traditional monitoring approaches (European Water Framework Directive 2000/60/EC-EU WFD and, in Switzerland, the Water Protection Ordinance-WPO) by using DNA metabarcoding techniques to complement classical proxies. The environmental DNA (eDNA) approach will make use of high-throughput sequencing (HTS) to analyse both specific bioindicators (such as diatoms, cyanobacteria, or fish) and a broader diversity (targeting bacteria and micro-eukaryotes). Pilot sites are lakes and rivers in the Alpine region, where traditional ecological assessment will be compared to eDNA inventories. In this context, protocols for sampling, DNA extraction, library preparation, and bioinformatics are formalized and are made available (e.g., via protocols.io or online videos). The main outputs of the project are (*i*) to provide guidelines for the integration of HTS results in the definition of ecological status, and (*ii*) to promote the transfer of eDNA tools for implementation of operational biomonitoring of lakes and rivers.

Status and highlights of the global DNA barcode reference library for marine peracarids (Crustacea: Peracarida)

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Background: Environmental DNA (eDNA) metabarcoding studies rely on reference libraries to accurately assign the sequence data obtained to the correct taxa. However, these reference libraries are sometimes small or lack significant information of the studied group. One notorious example is the superorder Peracarida, which is a highly diverse and common crustacean taxon in marine ecosystems around the world. Here, we review the status of the global DNA barcode reference library for marine peracarids. **Results:** A total of 6816 DNA barcodes were compiled in a BOLD dataset. Only barcodes with more than 500 bp, without codon stops or contaminations, and with the indication of the sampling location of the respective specimen were used. The dataset included specimens of the orders Amphipoda (458 species, 79 families), Isopoda (100 species, 25 families), and Tanaidacea (13 species, 5 families). The Atlantic Ocean is the one with the highest number of sequences/species and the Indian Ocean with the least. In total, the 571 peracaridean morphospecies were assigned to 797 BINs, with 160 morphospecies represented by two or more BINs and representing almost half the number of BINs (386). Moreover, just 9 morphospecies contributed 81 BINs. **Significance:** Although the superorder Peracarida is diverse and abundant across the world's marine environments, only 571 of the more than 10 000 known marine peracarid species are currently represented in BOLD by records fulfilling our selection criteria. Moreover, there is a large gap of representative species barcodes from most oceans. The high proportion of surplus BINs compared to morphospecies (additional 28%) confirms the importance of library-building efforts to unravel suspected hidden diversity and to contribute to tackling the different shortfalls in marine biodiversity knowledge. However, a substantial research effort is still required to further populate the peracarid reference library, in order to benefit from the full potential of DNA-based biomonitoring tools.

Prominent evolutionary divide between Macaronesian islands and nearby continental coasts in multiple peracarids (Crustacea): over 60 suspected new species

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Background: The marine realm is often seen as a continuous medium, without evident geographical barriers that could restrict the dispersal of marine organisms and gene flow. However, recent studies have shown deep genetic segregation in some peracarid species in the Northeast Atlantic, indicative of ecological and evolutionary mechanisms so far poorly understood. To investigate the suspected evolutionary divide between the populations from Macaronesia and nearby continental coasts (Iberian Peninsula and Morocco), we conducted a meta-species screening by examining DNA barcode differentiation among 23 peracaridean morphospecies present in both regions. **Results:** A total of 89 molecular operational taxonomic units (MOTUs) was diagnosed for these 23 morphospecies. Minimum and maximum conspecific inter-MOTU distances ranged from 3.4% (*Ampithoe ramondi*) to 26% (*Janira maculosa*). Macaronesia islands harbored more MOTUs than continental coasts (70 vs. 38), displaying 53 endemic MOTUs (vs. 23 of continental coasts) and with only 7 MOTUs shared between these two regions. Differentiation between continental and macaronesian populations were higher than 3% in all morphospecies. All the studied islands (except El Hierro) had private MOTUs, with the islands of La Palma (12) and Madeira (8) displaying the highest numbers. **Significance:** DNA barcode data uncovered an exuberant endemic diversity of peracarid fauna in Macaronesia, and a sweeping phylogeographic discontinuity between these archipelagos and the nearby and continental coasts. These findings call for a re-appreciation of the underlying evolutionary processes promoting diversity and segregation of these marine invertebrates in oceanic islands. They also emphasize the genetic heritage hosted by some unprotected areas in Macaronesia, highlighting the need to consider organisms with comparatively lower dispersal, and look at the fine-scale endemicity, in order to design more effective networks of marine protected areas. Such considerations will be particularly critical if identical segregation patterns are found in other, yet unscrutinized, marine invertebrates, particularly those dispersing through planktonic larvae.

Quantifying pollinator diversity along urbanization gradients in the Loire Valley

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Background: Pollination is a major ecosystem service required for 15%–30% of human food production and the reproduction of many plant species. One of the main anthropogenic causes of wild bee pollinators decline is the loss of natural habitats by extensive urbanization. This study aimed to assess the potential loss of pollinator diversity along urbanization gradients around three mid-sized cities (Orléans, Tours, and Blois) in the Loire valley (Central France) in the summers of 2017 and 2018. We collected flying insects using colored pan traps and identified them using a combination of morphology and DNA barcoding, focusing both on bees and Diptera. **Results:** Preliminary results based on bee samples suggest a significant negative effect of urbanization on the number of genera and species, irrespective of the city. One single specimen of each bee species was used to

compile a reference library of DNA barcodes for further species-level identification. **Significance:** This work will provide a detailed measure of the impact of urbanization on two main groups of pollinators (bees and flies) and will address questions about the ecological key factors for biodiversity conservation purposes in urban landscapes. Our reference library of wild bee barcodes in the Loire Valley is the first step towards the design of a biomonitoring program on the decline of pollinators in this area.

The SITE-100 project for site-based sampling and mitochondrial metagenomics of arthropods

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Background: The SITE-100 project is a collaboration of researchers conducting in-depth sampling of local sites (observatories) and standardised metagenomic sequencing of samples, to capture the great diversity of insects and other arthropods on Earth. The project addresses the problem that for many highly diverse groups of arthropods, species-level sampling remains unachievable. Instead, a phylogenetic approach based on mitochondrial metagenomics and reduced sampling from selected sites around the globe are used for the analysis of large-scale patterns of biodiversity. **Results:** To date, the approach has been trialled at high-diversity tropical sites. Several thousand mitogenomes have been produced primarily for Coleoptera (beetles) and placed in a well-supported phylogenetic tree. The tree also constitutes a phylogenetic framework for an evolutionary placement of unidentified barcodes and metabarcodes. Even a small number of sampled sites provided a large proportion of the global diversity of clades, corresponding to the genus or tribal level, indicating that most of phylogenetic diversity can be encapsulated by a limited set of study sites around the globe. **Significance:** The combination of site-based sampling and metagenomic sequencing provides a new powerful methodology for gaining an evolutionary perspective of global biodiversity. The resulting tree, if sampled sufficiently densely, is a highly informative framework for the much shorter sequences from COI (meta)barcoding, which have little phylogenetic information content of their own. The SITE-100 project makes available unified protocols for all aspects of site-based studies, from unified field protocols, to laboratory procedures for efficient mitogenome sequencing, to the bioinformatics pipelines and data compilations. We invite interested researchers to add to the existing sites and become part of the consortium, to improve the geographic coverage and grain size of the sampling.

The Nagoya Protocol — basic set up and implications for research

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The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity is an international agreement which entered into force on 12 October 2014. The bilateral default system under the Nagoya Protocol is based on an assumption that biodiversity-rich provider countries will regulate access to genetic resources. Researchers who use genetic material from different countries might need to fullfill several access requirements and(or) benefit-sharing requirements as part of the research project. The Party where the genetic resources are being utilized, e.g., Norway, has to make sure that genetic resources utilized within the Norwegian jurisdiction has been accessed in accordance with prior informed consent and that mutually agreed terms have been established, as required by the other Party. Research and development is a way of utilizing genetic resources. There is great diversity how countries have regulated genetic resources. Some countries regard the use of derivatives based on genetic material, such as products and the use of digital sequence information from genetic material, as utilization of genetic resources, while other countries do not. And many countries have free access, i.e., no regulation. This reflects that the Protocol gives a wide margin of discretion on this issue. For instance, Norwegian rules differ from EU regulation. The rapid technological development in the use of digital sequence information has created uncertainty on how the Convention on Biological Diversity and the Nagoya Protocol relates to digital sequence information. In my presentation, I will focus on the basics of the Nagoya Protocol, related processes, and potential challenges related to its implementation for research.

Menu à la MinION: real-time DNA sequencing for diet of large African mammals

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Background: Recent advances in genetic technologies engineered for portability allow sequencing while in the field for near-immediate results. However, despite the great need for this capacity for biodiversity, conservation, and disease-related research, challenges prevent routine deployment at sites that are extremely remote from home laboratories. To help push this technology forward, we used an Oxford Nanopore Technologies MinION sequencer and associated equipment for amplicon sequencing from feces, and targeted diet as a proof-of-concept question. In June 2018, we deployed our "Species from Feces Mobile" backpack-sized field kit in South Luangwa National Park, Zambia, which is >60 h travel time from our institution, focusing on nine large mammal species. We also tested and optimized the system with feces of jaguars (*Panthera onca*) and bobcats (*Lynx rufus*) with known diet as well as mock communities to assess detection capability, and compared amplicon sequencing results from second-generation (Illumina) and third-generation (MinION) sequencers. **Results:** During six days at our Zambian field site, we collected seven fresh African lion (*Panthera leo*) and leopard (*Panthera pardus*) feces, extracted DNA, sequenced the mitochondrial gene COI, confirmed vertebrate host, and identified prey species in the recent diet. From an additional 23 freshly deposited herbivore fecal samples, we used COI and ITS2 barcodes to confirm the vertebrate host while identifying the plant diet to genus-level resolution. MinION sequencing results were on par with those from an Illumina MiSeq. **Significance:** We evaluated the strengths and pitfalls of portable real-time sequencing as it currently stands via a project that brought together big cats and miniaturized sequencers, illustrating that portable amplicon sequencing to answer questions at remote sites is feasible even under sub-optimal reagent storage conditions due to extensive travel times. We detail shortcuts and alternatives for sample preparation, which decrease time and cost of field-based sequencing, and provide logistical recommendations for sequencing in the field.

DISCO: creating a research hub for eDNA research in California

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Background: The Diversity Initiative for the Southern California Ocean (DISCO) is a large research program at the Natural History Museum of Los Angeles County (USA) that aims to develop research and monitoring tools to enormously accelerate our ability to discover and investigate the marine invertebrate biodiversity of California's nearshore waters. DISCO is simultaneously generating and curating the estimated 25 000 DNA reference barcodes necessary to capture the marine invertebrate diversity of coastal California from 0 to 1000 m in depth as well as conducting numerous environmental DNA (eDNA) surveys of the nearshore across a range of habitats. Sampling techniques include sediment samples from rocky intertidal, sandy beach, and subtidal sites, and water samples including some inside the Port

of Los Angeles, one of the world's busiest shipping ports. These samples were analyzed using a variety of eDNA methods, including metabarcoding and qPCR-based approaches. **Results:** We have paired our eDNA sampling with a variety of traditional marine biosurvey techniques, such as benthic trawls and crowd-sourced citizen scientist photo observations on the iNaturalist platform. We will present the results of our eDNA survey and compare and contrast them with the results of these more traditional methods. Additionally, we will demonstrate how the choice of a particular DNA barcode reference database (e.g., BOLD, GenBank/EMBL, etc.) affects taxonomic assignment of sequence data from eDNA metabarcoding studies. **Significance:** Our work demonstrates the power of eDNA as a tool for increasing the amount of data that can be generated per unit effort and the necessity for robust regional DNA barcode reference databases to give biological meaning to eDNA metabarcoding results. We highlight the role natural history museums can play by catalyzing engagement between academia, citizen natural historians, and public agencies.

Dietary analysis of bat feces between rainy season and dry season

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Background: Tropical cloud forests have increased biodiversity. These environments experience an annual switch between rainy and dry seasons. With the change in rain amounts comes a change in food availability for bats, whether frugivorous or insectivorous. **Results:** We examined the feces of multiple individuals of two species of frugivorous bat as well as a variety of insectivorous bats to see if food choice changed between wet and dry season. We present here comparisons of the two seasons. **Significance:** Understanding food choices of bats allows regional coffee farmers to selectively plant or leave plants and trees the bats select for foraging. This kind of monitoring also may provide information on the presence of insects and plants that are not easily sampled.

Community dynamics and barcoding of protozooplankton in the Trondheim Fjord, Norway

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Background: Protozooplankton (micrograzers between 20 and 200 μm) has an important consumer role within the microbial loop and serves as an important diet component for higher trophic levels such as copepods and first-feeding fish larvae. Thus, protozooplankton (PZP) is considered as an important trophic intermediary between microbial and traditional food webs and has a key role in trophic upgrading. However, while current marine barcoding initiatives have a good coverage of photosynthetic protists and metazoans relevant in Norwegian waters, important representative groups of PZP such as ciliates and heterotrophic dinoflagellates are still lacking sufficient coverage. **Results:** In this study, we assess the seasonal abundance and composition of PZP in the Trondheim Fjord and produce DNA barcodes for the seasonally most abundant key species of ciliates and heterotrophic dinoflagellates. For this, we conducted isolation of live cells from seawater samples throughout the years 2018–2019 and conducted single-cell PCRs with primers for the 18S and COI barcode regions. **Significance:** The produced species-specific barcodes will contribute not only to reducing the barcode knowledge gap of PZP, but also to pushing forward the identification of PZP as prey in the gut contents of marine fish larvae and other metazoans, which will lead to a better resolution of marine food web interactions and dynamics.

Identification of the medicinal plants in *Aconitum* L. from China by DNA barcoding

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Background: DNA barcoding is a technique for species identification using a short DNA sequence. It is one of the most rapidly developing directions of biological studies in recent years. *Aconitum* L. consists of approximately 400 species and is widely distributed throughout the temperate regions of the north hemisphere. This genus is very richly represented in China; there are about 211 species, of which 166 are endemic. These species are commonly used in China for medicinal purposes, but species identification is also crucial. **Results:** In this study, 183 individuals from 42 medicinal species of *Aconitum* were sequenced for seven proposed DNA barcodes (ITS, rbcL, matK, psbA–trnH, psbI–psbK, tabE–tabF, trnE–trnY) and evaluated with four analytical methods (NJ, PWG, BLAST, and Distance). The varied approaches have different species identification powers. With the Distance analysis, ITS + matK + psbA–trnH and ITS + matK + psbA–trnH + psbK–psbI combinations showed the highest discrimination ability (both 60.65%) among all combinations. With the PWG analysis, psbK-psbI has the lowest discrimination ability (11.90%). As a single barcode, ITS showed the highest discriminatory power (52.38%), and any combination of ITS with another barcode could improve discrimination power. Furthermore, the number of variable sites, informative sites, and intraspecific distances of ITS were the highest among all the single barcodes, and it was the most effective barcode for *Aconitum* species identification. The most effective two-region barcode was ITS + psbA–trnH (54.64%), and the most effective three-region barcode was ITS + matK + psbA–trnH (60.65%). The relatively low DNA barcoding discrimination in these medicinal species (11.90%–60.65%) might possibly be attributable to the complex speciation, recent radiations, and hybridizations of this genus. In addition, seven mislabeled samples were found and corrected, and one cryptic species *Aconitum wumengense* J. He & E.D. Liu, sp. nov. was described and illustrated based on this study.

From COI metabarcoding to metaphylogeography: finding the optimal way to get robust haplotype information from raw HTS data

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Background: The natural variability of COI makes it the marker of choice in population genetics and phylogeography studies. The exploitation of COI metabarcoding datasets for intraspecies patterns of genetic variation makes it possible to analyse phylogeographic features for hundreds of species at one time—a field which we name metaphylogeography. The key to implement this approach is to separate erroneous sequences from real intra-MOTU variation. This requires a fine-tuned denoising algorithm to be able to distinguish low-frequency naturally existing haplotypes from artefacts generated by sequencing errors. **Results:** We calculated the changes in entropy at the different codon positions and the changes in the residual variance of AMOVA models to find optimal filtering parameters in COI metabarcoding datasets. These two independent criteria, coupled with co-occurrence patterns of sequences, can meaningfully guide the cleaning of metabarcoding datasets for metaphylogeography. Using a dataset of community DNA from benthic littoral communities from the Mediterranean and Atlantic seas, we developed a two-step cleaning procedure consisting of a denoising within MOTUs followed by a minimal abundance filtering. Reassuringly, these two independent information-content criteria provided similar optimal parameter values for the denoising and abundance

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.

DNA barcoding marine fauna with NorBOL — current status

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Background: The Norwegian network NorBOL has obtained samples of marine fauna from a geographical range defined approximately by 71°N 54°W, 85°N 130°E, 49°N 7°E, and 55°N 14°E, spanning part of the Arctic Sea, Norwegian Sea, North Sea, Skagerrak, and Øresund. Several Norwegian and Swedish research institutions have been involved in sorting, identification of specimens, tissue sampling, and sample documentation, whereas most of the sequencing was performed at CCDB, University of Guelph, Canada. **Results:** The campaign has produced 9600 species records with pictures of invertebrates and 530 of fishes. Moderate to relatively poor sequencing success for some taxa, with an average of 60%, returned totally 5590 cytochrome c oxidase subunit I (COI) sequences of invertebrates. Totally 4380 of these, representing 1550 species, have been approved as barcode compliant. From 152 species of fishes, including freshwater samples, sequencing returned 362 barcode-compliant sequences. Analyses of distances, based on sequences 400 bp or longer, showed that invertebrates had a mean intraspecific divergence of 2.93% and that 60% of the species were less than 2% divergent. By contrast, intraspecific divergence in fish was only 0.03%, with 90% of species less than 2% divergent. While extreme intraspecific divergence was estimated in many cases, some can be explained by misidentification. Totally, 1550 morpho-species of invertebrates were assigned to 1642 BINs by the Barcode of Life Data Systems (BOLD), indicating cryptic species. **Significance:** The records constitute a substantial contribution to a barcode library of North Atlantic fauna. High intraspecific divergence and considerable proportions of BIN discordance call for continued efforts to improve the taxonomic quality of the database. Nearest neighbour analyses, with extreme cases of 64.18% distance to the nearest neighbour, are indicative of missing species representation in the database.

Using metabarcoding to characterise contemporary and historic lake communities in New Zealand

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Background: New Zealand's lakes are nationally significant freshwater bodies that provide critical ecosystem services and hold high cultural importance. However, informed management decisions cannot be made due to a paucity of knowledge. Water quality data exist for fewer than 5% of New Zealand's 3800 lakes (>1 ha), and biodiversity data are only available for a subset of these lakes. In this study, we are collecting sediment cores, surface sediment, and water samples from 10% of New Zealand lakes. Metabarcoding (16S rRNA, 18S rRNA, and COI genes) is being used in parallel with a suite of traditional and paleolimnological techniques to characterise contemporary and historic lake biodiversity across a wide range of phyla. **Results:** Side-by-side morphological and metabarcoding analysis shows strong correlations in species identification for selected organisms (e.g., macroinvertebrates). Metabarcoding is now allowing the characterisation of contemporary lake biodiversity at much greater spatial scales. The application of metabarcoding to characterise historic changes in lake communities is revealing new insights into past biodiversity and drivers of change. For example, our data have highlighted the impact of introduced fish on native communities and have allowed us to explore community responses to natural and anthropogenic disturbances. **Significance:** Environmental DNA-based methods, which enable rapid and cost-effective biodiversity assessments, will assist in prioritising management and mitigation efforts. This project will provide data that can be used in paleolimnological investigations to help elucidate lake environmental histories and establish evidence-based restoration targets.

Optimization of molecular methods involved in the eDNA metabarcoding of benthic macroinvertebrate communities

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Background: The use of benthic macroinvertebrate taxa as bioindicators of ecosystem change is a common practice in the field of biomonitoring. As genomic tools such as environmental DNA (eDNA) metabarcoding become more accessible, it is important to have the most effective methods for producing high-quality data. Benthic samples are inherently "messy" as a result of riverbed substrate introducing molecules which can inhibit downstream genomic applications. Our study investigates the impact of DNA extraction method on PCR efficiency, as well as introduces optimized PCR protocols to reduce the amount of chimeric, or otherwise spurious, sequences produced during amplification. To establish this, benthic macroinvertebrate communities were sampled in six locations across the city of Waterloo (Ontario, Canada). **Results:** When comparing three extraction protocols, Qiagen's DNeasy PowerSoil kit was found to yield the highest quality DNA (assessed by nanodrop values, and confirmed by the strength of gel bands after PCR amplification). Next, by modifying aspects of our PCR protocol we were able to establish optimal conditions for assessing benthic macroinvertebrate biodiversity in our system due to a reduced number of PCR cycles and the inclusion of technical replicates. **Significance:** By using a DNA extraction protocol which includes secondary steps to remove PCR inhibitors with greater efficiency, we were able to increase the quality of the DNA which led to straightforward PCR amplification, requiring no troubleshooting. We were also able to generate higher-quality sequences, with greater biological signal while reducing noise. These factors combined can help researchers make the most of their sequencing runs, potentially allowing for a greater proportion of sequences to be assigned taxonomy. In turn, this information can aid in the biological inferences derived from sequence data, such as a greater ability to classify ecosystems impacts.

Rapid detection of all twelve CITES-listed shark species by loop-mediated isothermal amplification

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Background: Sharks have been overexploited to meet the growing demand for shark fins and meat, threatening dozens of shark species. Shark species have been listed in Appendix II of The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 2000, and 12 shark species are now CITES-listed. Species-level identification is essential for law enforcement and fisheries management. Shark fins entering international trade are usually skinned and bleached, and cannot be identified morphologically. DNA barcoding has been adopted for identification of CITES-listed shark species, but it is still too laborious and time-consuming for some customs officers, who have to screen up to thousands of shark fin pieces in one cargo. A rapid and accurate identification method for all 12 CITES-listed shark species has to be in place for governments of all Parties to CITES. **Results:** Species-specific loop-mediated isothermal amplification (LAMP) assays and species-specific end-point PCR assays were developed for all 12 CITES-listed shark species, with an internal control for all shark samples. Specificity of all assays was tested on 291 shark samples, covering 93 shark and related species from 7 orders and 26 families. The limits of detection for the end-point PCR assays are

0.2–10.0 ng/μL. LAMP assays are more sensitive and rapid, with limits of detection ranging from 0.2 to 5.0 ng/μL and a reaction time of 60 min. **Significance:** This is the first species-specific identification method that covers all 12 CITES-listed shark species, including the oceanic whitetip shark and whale shark, for which a rapid species-specific detection method was previously unavailable.

Medicinal plant DNA barcoding system of Changbai Mountain National Reserve

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Background: Changbai Mountain is the most completely preserved ecosystem with greatest biodiversity at the same latitude of the northern hemisphere, and also the largest medicinal plant repository in Northeast Asia. However, several human activities infringe on the biodiversity of the reserve and lead some species to become extinct in the wild. Consequently, the biodiversity of the reserve has been threatened and should be protected in case of the loss of wild resources. **Results:** A total of 1904 samples, belonging to 689 species, 403 genera, and 107 families, were involved to construct the medicinal plant DNA barcoding system of Changbai Mountain National Reserve. In total, 1763 and 1692 sequences belonging to ITS2 and psbA–trnH regions were obtained and included in the database. With the combination of botanical characteristics, ecological habitat, and molecular information, this system provides the public with the first multifunction searching tool based on a national reserve with the following functions: (*i*) basic information about the Changbai Mountain National Reserve; (*ii*) query and retrieval of the detailed characteristics of the medicinal plant species in Changbai Mountain, such as Latin and Chinese names, morphological characteristics, medicinal effects; and (*iii*) DNA barcoding identification of unknown samples based on the Basic Local Alignment Search Tool (BLAST) algorithm. **Significance:** This system can provide researchers and government regulators the general overview, ecological habitat information, and DNA barcodes of the medicinal plants in Changbai Mountain and will be a powerful tool for the biodiversity conservation of Changbai Mountain National Reserve.

Selection of medicinal plants using DNA barcoding and chemical profiling: an example of Icelandic *Huperzia selago*

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Background: Plants in the family Lycopodiaceae produce bioactive lycopodium alkaloids (LAs). In particular, the alkaloid huperzine A (hupA), which was first isolated from *Huperzia serrata*, is a potent acetylcholinesterase inhibitor, and it has been suggested for the treatment of Alzheimer's disease. Therefore, it is interesting to investigate other closely related *Huperzia* species. This study focused on Icelandic *Huperzia selago* and aimed to explore the correlation between genetic diversity and hupA contents. **Results:** Three chloroplastic DNA barcodes (e.g., rbcL, psbA–trnH, and matK) were amplified and sequenced to assess the genetic diversity of Icelandic *Huperzia selago*; the contents of hupA and diversity of LAs were determined by high-performance liquid chromatography coupled to photodiode array detector (HPLC-PDA) and ultrahigh-performance liquid chromatography coupled to mass spectrometry (UPLC-MS). In total, there are three genotypes of *H. selago* in Iceland. Interestingly, genotype three contains a significantly higher amount of hupA than genotype one, while the intermediate genotype two shows a broad content of hupA. Principal component analysis (PCA) of UPLC-MS fingerprints also shows that each genotype tends to have a unique alkaloid profile. A PCA loading plot reveals that hupA drives the differentiation of genotype 3 from the other genotypes. Thus, we suggest that genotype 3 should be a good alternative source for hupA production. **Significance:** This study highlighted the importance of integrating DNA barcoding and chemical profiling in identifying the plant taxa of higher pharmaceutical importance.

Species status, delimitation, and phylogenetic relationship of three *Altica* flea beetles

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Background: The Asian species *Altica cirsicola, A. fragariae*, and *A. viridicyanea* (Coleoptera: Chrysomelidae) are broadly sympatric and morphologically highly similar but feed on distantly related host plants. **Results:** Molecular phylogenetic analysis with limited sampling suggested they are likely to be each other's closest relatives. Cross studies showed that their post-mating isolation is incomplete, and interspecific hybridization among them could be generated under laboratory conditions, further indicating their close affinity. Based on our studies, the full species status of these three species was supported by different genitalia structure, host plant specialization, assortative mating, and species-specific cuticular hydrocarbons. Phylogenetic analyses based on both a single mitochondrial gene and the whole mitogenome data showed clear interspecific divergences of *A. fragariae* from the other species, but *A. cirsicola* and *A. viridicyanea* were not distinguishable by distance-based or tree-based methods of species delimitation due to nonmonophyly relative to the morphologically defined entities. However, the species delimitation of *A. cirsicola* and *A. viridicyanea* can be achieved by a nuclear marker, ITS2. The phylogenetic relationships among them are still confused; different relationships were inferred depending on the gene used. **Significance:** The confused relationship due to mito-nuclear discordance was possibly affected by interspecific introgression. We suggest that wide sampling and more nuclear markers are required to unravel the true speciation and demographic history of these three *Altica* species.

High-throughput sequencing accelerates the process of barcoding of life, from COI to mitogenome

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Background: Over the last decade, the rapid development of high-throughput sequencing (HTS) platforms has accelerated species description and assisted morphological classification through DNA barcoding. However, constraints in barcoding costs and incomplete databases led to unbalanced efforts, which prevented accurate taxonomic identification for biodiversity. In addition, investigation of phylogeny and biodiversity now requires multiple genes from the mitogenome or the entire mitogenomes to solve the problems for which a single gene does not perform well. We present a series of approaches that can achieve standard barcode sequences (COI) and mitogenomes from HTS platforms, including widely adopted 150 bp paired-end reads from Illumina and BGISEQ sequencing, and long reads from Pacific Biosciences (Pacbio) Single Molecular Real-Time (SMRT) sequencing and the 400 bp single-end reads from BGISEQ-500. **Results:** We evaluated the performance of our methods by comparing it to results from Sanger-based sequencing. It showed that HTS approaches can retrieve accurate full-length reference barcodes efficiently and with a higher success rate, at about one-tenth of the current cost for Sanger sequencing. For the whole mitogenome, our one-stop solution (MitoZ) can recover more full-length mitogenomes with higher accuracy than other available mitogenome assemblers. **Significance:** Our approaches can produce standard full-length barcodes cost-efficiently, which will advance DNA-based species identifica-

tion for various ecosystems and improve quarantine biosecurity efforts, enable construction of comprehensive barcode libraries for local fauna, lead to a feasible direction for DNA barcoding global biomes, and build a bridge to the barcoding world of the mitogenome-based system.

Molecular biosurveillance for species of regulatory interest using a high-concentration salt solution

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Background: The introduction of live wood-boring insects into Canada is concerning due to their ability to flourish in our forests and destroy hardwood trees. Biosurveillance programs that can provide early warnings are essential to reduce the impact of these species, and improved protocols to increase the efficacy, cost-effectiveness, and the speed of detection are needed. A biomonitoring program using Lindgren funnel insect traps with a high-concentration salt (NaCl) solution (HCSS) was conducted at two park sites in Ontario. Trap specimens were removed and stored for later morphological identification, and HCSS samples were retained for molecular analysis. Environmental DNA (eDNA) was filtered from the HCSS, extracted, amplified, and sequenced using an Illumina MiSeq platform. **Results:** High-quality amplicons representing a large portion of the animal barcode region (~470 bp) were obtained. Bioinformatic analyses were performed using Geneious and the mBRAVE platform. An average 199 415 reads per sample were obtained from our sequencing efforts and used in further analyses. Paired-end reads were merged using Geneious, with an average of 71% of the sequences retained per sample. Sequence reads not able to be merged were discarded from further analysis. Merged sequences were analyzed using mBRAVE and resulted in an average of 261 matched BINs, with an additional average of 1250 operational taxonomic units (OTUs) per sample. Our results included target taxa, with members of indigenous longhorned beetles (Cerambycidae) detected in all samples. In addition, we identified multiple species of Canadian regulatory concern in the HCSS from numerous traps. **Significance:** This work details an inexpensive trapping and metabarcoding protocol that provides long, high-quality sequences necessary for use in regulatory applications. The success of the HCSS method provides a valuable alternative to ethanol-based collections, which can be challenging to implement due to evaporation, laws and regulations governing the use of alcohols, and concerns about public exposure and tampering.

P.E.M.A.: a pipeline for environmental DNA metabarcoding analysis

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Background: Metabarcoding, the combination of high-throughput sequencing and DNA-based species identification, has gained ground over the last years. Inevitably, a plethora of tools has been developed for the analysis of such data; however, a pipeline implementing selected and reliable tools for all the necessary analytical steps and allowing the user to have complete control of the whole analysis is still lacking. **Results:** In an attempt to address this issue, a P.E.M.A. (pipeline for environmental DNA metabarcoding analysis) was developed for two marker genes, 16S rRNA and COI. The required inputs are .fastq files and a file with the user-specified analysis parameters. P.E.M.A. returns a molecular operational taxonomic unit (MOTU) table with the classification of the taxa found and their relative abundances in each sample. For 16S, further analysis of the OTU-table is also possible. For COI, two different clustering algorithms can be selected (CROP and SWARM), while for 16S data, both alignment- and phylogenetic-based taxonomy assignments are supported. Checkpoint files are created after each step, allowing multiple runs of similar analyses to be performed by changing only the parameter needed and resuming the analysis from the corresponding checkpoint. P.E.M.A.'s efficiency and accuracy was validated against publicly available datasets. For the 16S dataset, P.E.M.A. retrieved 4457 OTUs compared to the 7050 reported in the published study. For the COI dataset, P.E.M.A found 81 species, while the published study reported 73 species-level MOTUs. **Significance:** The novelty of P.E.M.A. lies in the facts it is performed in an uninterrupted workflow, is user customizable and high-performance computing (HPC) compatible, and requires low installation effort. P.E.M.A. has been containerized into a Docker image, which makes it compatible with all types of operating systems. It is also available as a Singularity image for implementation in HPC environments. The time-efficiency, ease of use, and quality of results suggest that P.E.M.A. is a valuable tool for accurate eDNA metabarcode analysis.

Inventorying Merbok River fish through DNA barcoding: developing a local database of metabarcode studies

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Background: The performance of DNA barcoding was tested to assess the species diversity of local fish catches in Merbok River, which is located in Kedah, Malaysia. The study was designed to examine the reliability of the DNA barcoding method as compared to morphological characters for species-level identification through conventional identification. This is of critical importance due to the fairly limited taxonomic studies on fishes in this country. This study also would greatly contribute to the development of a local barcode database that can be utilized in sympatric metabarcoding analysis. **Results:** Preliminary data analyses revealed a total of 11 orders, 44 families, and 54 species that were sequenced (barcoded) for a 655 bp region of the mitochondrial cytochrome c oxidase subunit I gene (COI). Most species were represented by multiple specimens, with mean sample size of 3 samples per species and a total of 154 sequences generated. In addition to the barcode-based species identification system, phylogenetic relationships among the species identified have also been attempted. **Significance:** The present study provided evidence that fish species can be efficiently identified through the use of DNA barcoding and that the present COI library can be used for subsequent applications in metabarcoding studies.

DNA barcoding assessment of commercial fishes in Malaysian waters: establishment of the first large-scale national fish barcode database

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Background: In many regions of the world, including Malaysia, fish stocks are being exploited without much taxonomic assistance. It is impossible to develop conservation plans and long-term management without knowing what species are involved. The basis for sound resource management is the ability to define the resource correctly. Recently, heads are turning to molecular aid for the rapid identification of organisms. It has been used in assisting fisheries management for long-term sustainability and for improving ecosystem research and conservation. In this study, we had demonstrated that DNA barcoding can be employed to gain new knowledge into a multi-species capture fishery, revealing the likely presence of an unrecognized fish

species and the undocumented exploitation of others. **Results:** A total of 107 species, 69 genera, 36 families, and 10 orders of commercial fish specimens was recovered and barcoded, and it is visibly shown that genetic divergence increased with higher taxonomic rank: 0%–19.75% within species, 3.86%–22.47% within genera, and 7.91%–26% within families. **Significance:** The diversity documented here will be a useful resource for future researchers and managers seeking accurate information on the species composition of the commercial marine fisheries and will ultimately aid the formulation of effective conservation plans.

In one fell swoop: co-detection of nontarget DNA with third-generation DNA barcodes

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Background: Adoption of third-generation sequencing for assembly of reference libraries relies largely on the capacity of single-molecule real-time (SMRT) sequencing to generate long reads that produce high-fidelity circular consensus (CCS) results. In particular, the Sequel platform from Pacific Biosciences has proven its utility in generating highly, accurate full-length DNA barcodes in a high-throughput fashion. Because a singular template is being analyzed during SMRT sequencing, any amplicon from a secondary nontarget DNA contributor may produce a barcode conflict within an individual sample. Resolving such conflicts requires understanding of the underlying factors that drive formation of multiple consensus sequences from a single-source DNA extract. Yet, there is very limited information about the frequency of these conflicts and even less knowledge about the source of nontarget DNA barcodes. **Results:** This study explores factors that contribute to co-detection of multiple DNA templates from an individual specimen based on the results of one million samples that underwent barcode analysis on a Sequel platform at CBG. Although the samples represented broad geographic distribution, the taxonomic coverage was limited to taxa commonly captured in Malaise traps, i.e., arthropods, predominantly from five insect orders (Diptera, Hymenoptera, Coleoptera, Hemiptera, Lepidoptera). While the overall success (a sample with a sequence) was edging towards 90%, at least 10% of these samples produced multiple consensus sequences with divergence over 2%, yet for the overwhelming majority of these the most abundant variant corresponded to the target organism. **Significance:** This study confirms the reliability of third-generation barcodes generated through SMRT sequencing and will aid in developing validation procedures for reference libraries with limited information (limited taxonomic information, possible lack of vouchers, absence of voucher images). This study also demonstrates the value of SMRT sequencing for studying interspecific interactions and for the discovery of taxa that otherwise could be overlooked.

Genetic diversity of *Gomphocarpus sinaicus* Boiss. within and between wadi systems of the St Katherine Protectorate (South Sinai, Egypt)

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Background: The genetic variation of the plant *Gomphocarpus sinaicus* Boiss. (Asclepiadaceae) was assessed within and between populations from seven wadis (valleys) in the mountainous area of the St Katherine's Protectorate, Sinai, Egypt. The following DNA (RAPD) primers were used: OPH-03 (AGACGTCCAC), OPH-12 (ACGCGCATGT), OPH-18 (GAATCGGCCA), OPH-19 (CTGACCAGCC), and OPH-20 (GGGAGACATC). The results of banding patterns of DNA fragments generated by each primer were recorded. **Results:** Eighty-nine DNA fragments were generated from the seven sites. The total number of bands scored per primer ranged from 13 (OPH-20) to 19 (OPH-12). The size of the amplified fragments ranged from 225 to 3070 bp. A neighbour-joining tree showed that most individuals from a given population tend to cluster together, and are therefore more genetically similar than individuals from different populations. Low levels of diversity were present within populations, which reflect high gene flow within each valley. There were highly significant differences between the populations of *G. sinaicus* from the seven sites, using a one-way ANOVA ($F_{6,65} = 5.823$, $P < 0.0001$). Furthermore, there was a significant positive correlation between genetic distance and geographical distance ($r = 0.549$, $n = 7$, $P < 0.05$) between the populations of *G. sinaicus* in different valleys. **Significance:** The highly significant values of genetic differences reflect limited interpopulation gene flow that could be attributed to the mountainous nature of the St Katherine's Protectorate acting as a natural barrier to gene flow and leading to genetic divergence among different wadis.

Fishing for fish parasites: establishing a DNA-barcoding protocol for reliable species identification of *Gyrodactylus* (Monogenea, Plathyhelmithes) ectoparasites

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Background: Ectoparasitic flatworms of the genus *Gyrodactylus* (Monogenea, Platyhelminthes) are characterized by high host specificity. The genus contains an estimated number of 20 000 species worldwide, with only a little over 400 formally described to date. Little is known about their distribution and diversity in Austria. Since their discovery in 1832, taxonomy has been traditionally based on host species identification and the morphology of the hook apparatus. Existing molecular markers (ribosomal ITS) often lack resolution for species discrimination, and further (molecular) investigation is currently hampered by the lack of reliable generic barcoding primers for the genus. In the framework of the ABOL (Austrian Barcode of Life) initiative, we are working towards establishing generic primers and efficient protocols to ultimately reveal and characterize the actual diversity of *Gyrodactylus* species on Austrian fish species. **Results:** In the course of the Austrian Barcode of Life (ABOL) initiative, we developed new PCR primers for the amplification of the entire mitochondrial COI gene. We have successfully amplified the fragment for 35 *Gyrodactylus* species and are in the process of verifying the primers' generic nature further on a wide range of taxa. We present our first data obtained using the portable MinION sequencing platform (Oxford Nanopore), as well as discuss challenges and future directions. **Significance:** With the universal barcoding primers established by our approach, characterizing the tremendous *Gyrodactylus* diversity should be straightforward (not only in Austria). Even though *Gyrodactylus* species are not of direct human health concern, a number of species are known to cause huge economic losses in the aquaculture and fishing industries. In addition, with the increasing intentional and unintentional stocking and translocation of fish species, and thus also their parasites, some *Gyrodactylus* species might increasingly pose a threat to local fish populations, and reliable identification of the parasites is required to take timely measures.

Rapid and accurate species-level identification of opium poppy

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Background: *Papaver somniferum* L., commonly known as opium or oilseed poppy, is an ancient crop and medicinal plant cultivated for its edible seed as well as for the production of opium, the source for important pharamaceutical drugs including morphine, thebaine, codeine, papverine, and noscapine. To find a rapid and efficient way of drug ban, it is urgently needed to establish a molecular identification system to identify poppy seeds and seedlings. **Results:** *Papaver somniferum* and its eight closely related species, with 60 individuals selected for analysis, were sequenced using a genome skimming approach. We evaluated the species discrimination of rDNA and the

plastid genome. *Papaver somniferum* was clearly discriminated from its allied species by reference to the sequences of rDNA (710 bp) and plastid genome (152 931 bp). We also identified microsatellite (SSR) loci in the resulting sequences. 254 loci with microsatellite motifs were revealed. Initially, we designed primers for 121 loci, which were tested for PCR amplification and length variation on 60 individuals of *P. somniferous* and its closely related species. Finally, two validated SSR loci (PSS20 and PS120), generating clear strips of ~300 and ~100 bp, respectively, successfully amplified unique SSR segments in *P. somniferum*. AtpB200, which can produce a strip of ~200 bp in most angiosperm species, was selected as an internal control primer. Furthermore, a multiplex PCR protocol to get segments of those three loci in one reaction was established, and it could identify opium poppy accurately in 6 h. **Significance:** Genome skimming sequencing resulted in the completely assembled plastid genome, rDNA, and nuclear SSR loci for *P. somniferum*. Using the plastid genome, rDNA, and SSR loci, *P. somniferum* can be clearly and accurately discriminated from its closely related species.

Pollen preference and diet diversity of eastern honey bees revealed by genome skimming

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Background: Identifying variations in pollen transportation and consumption is often challenging for insect pollinators because pollen classification is difficult and metabarcoding of pollen mixtures is rarely quantitative. Thus, systematic examination of pollen load and diet among and within pollinator species has been scarce. The lack of basic knowledge on insect–pollen interactions prevents us from understanding some of the most essential ecological questions. For instance, is the introduced western honey bee (*Apis mellifera*) presenting an ecological challenge to the endemic eastern honey bee (*Apis cerana*)? In this study, we examined the efficacy of genome skimming (shotgun sequencing of pollen mixture without PCR amplification) coupled with extended chloroplast reference genes in pollen quantification. We used the new pipeline to detect variations in pollen compositions among honey bee colonies, as well as between individual bee diets. In addition, we compared pollen transportation diversity between the western and eastern honey bees that shared similar floral habitats. **Results:** We demonstrated that genome skimming was highly sensitive and accurate in detecting plant species from pollen mixtures, while revealing a high correlation between shotgun reads and pollen counts. Enabled by this new pipeline, we found significant variations between individual bee diets and pollen compositions among different colonies. In contrast to common beliefs, we showed that the western and eastern honey bees shared largely overlapping pollen loads under similar habitats, suggesting strong ecological competition when raised close together. **Significance:** Pollen genome skimming is able to produce better quantitative information for pollen composition, especially when chloroplast references are well represented for the local flora. We expect that pollen quantification will help to fine-tune pollination contributions between and within insect pollinators and to elucidate interactions among various pollinators.

German Barcode of Life 2 (GBOL2) — diatom DNA barcoding and eDNA metabarcoding in the context of the EU Water Framework Directive (2000/60/EC)

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Background: The GBOL2-Project, funded by the German Federal Ministry of Education and Research, is the second project phase of GBOL, running from 2016–2018. It focuses on the extension of DNA barcode reference libraries to integrate frequent, common, and indicator species; health-relevant and invasive organisms; as well as agricultural pests. The sub-project at the BGBM aims to obtain DNA barcodes of the 400 most important indicator species of the 1700 diatom species expected to live in German limnic waters. All DNA barcodes and correlated information will be made publicly available via, e.g., INSDC, BOLD, and GGBN. DNA stocks will be deposited in the BGBM DNA Bank connected to voucher specimens deposited at Herbarium Berolinense (B). **Results:** One thousand clonal strains have been established so far, belonging to 50 genera and 250 species. DNA barcodes have been obtained for more than 95% of those 1000 strains for both of the target barcode regions (18S V4 rDNA and rbcL). The morphology of each strain has been documented with light microscopy and scanning electron microscopy images. This barcode reference library uses the EDIT platform for cybertaxonomy, allowing the possibility to assign environmental sequences gained from environmental DNA (eDNA) metabarcoding. Additionally a fully automated, modular platform for eDNA metabarcoding data evaluation (https://github.com/sproft/MetBaN) has been developed. **Significance:** This project shows a best practice use case for documenting and displaying barcode reference libraries, environmental data, and eDNA data. This will contribute in the development of a sequence-based, time- and cost-efficient method to analyse diatom community composition in environmental samples via eDNA metabarcoding for water quality assessments and biomonitoring. In addition, all samples and vouchers will be made available through the GGBN Data Portal (http://www.ggbn.org).

Metabarcoding reveals seasonal diversity patterns in aquatic macro- and microorganism communities and the impact on biomonitoring aspects

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Background: Recurrent freshwater bio-assessments are important to monitor trends in ecological status and biodiversity and to validate the efficiency of conservation measures. The diversity and abundance of macroinvertebrates is a widely used indicator in such assessments worldwide and especially in the EU Water Framework Directive (WFD). Similarly, diversity patterns of microorganisms (typically diatoms) are used, yet the majority of other microbial organisms (bacteria, protists) are typically not included in assessments despite that they hold strong bioindication potential. This is because, with the exception of diatoms, microbiota are difficult to determine morphologically. In the present study, we applied DNA metabarcoding to both stream macroinvertebrates and microbial taxa. The first aim was to determine the impact of natural seasonal fluctuations in macroinvertebrate communities on assessment results. The second aim was to assess diversity patterns of bacteria and protists between seasons and compare their bioindication potential across differently impacted sites with macroinvertebrates. **Results:** Macroinvertebrates and microorganisms were sampled biannually (two years, spring/autumn) at six locations of a mid-sized mountain stream in Germany (River Sieg). Metabarcoding revealed strong seasonal fluctuations in community composition for all investigated taxonomic groups. For macroinvertebrates, this had minor influence on the determined ecological status, which was largely congruent with estimations based on morphological identifications. Diversity patterns of microorganisms indicated similar ecosystem conditions as those assessed through macroinvertebrates, but had an order of magnitude more taxa/operational taxonomic units. **Significance:** This study validates further the potential of metabarcoding to reliably infer community composition of macroinvertebrates as well as for prokaryotic and eukaryotic microorganisms. Furthermore, it indicates the ecological quality status weakly fluctuates depending on sampling season, yet shows a high congruence for macroinvertebrates between traditional and genetic methods. This finding is encouraging given the comparability of DNA-based and classical approaches in the context of national and international assessments, such as in the context of the WFD.