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ABSTRACTS

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Barcoding natural history collections with special attention to type material: challenge to taxonomy, nomenclature, and species distribution patterns. Case study of Palearctic voles and lemmings

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Background: The proper use of species names depends entirely on the process of verifying whether additional specimens are conspecific with the specimen with which the species name is associated. Thus DNA barcoding of museum specimens is of paramount importance in the elucidation of complex nomenclature issues. It is also a priceless source of material that was gathered during preceding centuries in sites hardly accessible today due to political or economic reasons. **Results:** DNA was successfully isolated and amplified in 32 type specimens of voles and lemmings (subfamily Arvicolinae, Rodentia) from the collection of the Zoological Institute in Saint-Petersburg, including samples collected as early as 1826. New insights from museum specimens dramatically change conventional ideas on lemming species (genus *Lemmus*) distribution and taxonomy. The attribution of all lemming specimens from vast areas of western Beringia, including Kamchatka Peninsula, to *L. amurensis* was erroneous. After genotyping a number of samples from the generic group *Microtus*, some of them were redefined. Analysis of the *Neodon juldaschi* holotype put a final dot in the nomenclature issue, and this species should be reclassified as belonging to genus *Blanfordimys*. **Significance:** Barcoding of old museum specimens allowed the finding of *L. trimucronatus* on the west coast of the Kamchatka peninsula, which is novel and unexpected. This finding adds the new species to the fauna of the peninsula. Genetic examination of *L. amurensis* holotype and other museum material assigned to this species proves that this taxon has a very limited range. Previous belief of its wide distribution and its status as Least Concern (LC) was based on incorrect species identification. The only sustainable population of this species remains in the area of the Chulman River, South Yakutia, and the conservation status of the Amur lemming undoubtedly needs to be reclassified as Vulnerable/Near Threatened (VU/NT).

DNA barcoding and the molecular clock in animals

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Background: The molecular clock is a fundamental tool in evolutionary biology. However, there remain important gaps in our knowledge regarding systematic variability in rates of molecular evolution across taxa and environments. Standardized DNA barcode sequences provide an outstanding public resource for research in molecular evolution and for the future application of molecular clock calibrations in ecological and evolutionary research. Here, we review our recent research into both absolute and relative rates of molecular evolution in the animal barcode region of cytochrome *c* oxidase subunit I (COI), spanning six diverse phyla, with particular emphasis upon insects, marine macroinvertebrates, and bony fish. **Results:** In most taxonomic groups examined, variability in rates of molecular evolution was relatively constrained among close relatives, suggesting that taxon-specific molecular clocks are likely to provide accurate divergence date estimates in many cases. Nevertheless, univariate and multivariate analyses, accounting for phylogenetic relationships, revealed several traits that were significant predictors of molecular rates. While inhabiting different environments was weakly predictive of rates, traits associated with differences in population structure and breeding mode were more strongly associated with rates. These results suggest that, in general, variability in the fixation rate—rather than variability in the mutation rate—is the stronger driver of differences in COI rates in animals. **Significance:** Overall, our results suggest a promising future for obtaining accurate date estimates for evolutionary events using DNA barcode sequence data, over a useful range of node ages up to the divergence threshold where transitional mutations no longer accumulate at a linear pace. Multivariate models are helping us to understand when correcting the molecular clock for traits and environments will be most suitable. These developments are expected to open new research avenues, such as understanding the impact of prior environmental and geological changes upon the diversity and distribution of biodiversity.

Entomological surveillance using DNA barcoding identify presence of *Lutzomyia verrucarum* sandfly (Diptera: Psychodidae) in leishmaniasis endemic community in Mexico

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Background: Continuous endemicity and re-emergence of vector-borne diseases in Mexico have called for a new approach to vector control programs including regular surveillance. Correct and accurate identification of vectors is essential for a successful surveillance program. This study uses DNA barcoding under the Mexican Barcode of Life (MexBol) project to improve vector surveillance and accurately delineate arthropod vector diversities including sandfly for leishmaniasis control in Mexico. In October and November 2016, sandflies were collected from different regions in Quintana Roo, Mexico, where leishmaniasis is endemic, using CDC light and Shannon traps. This project formed part of the health ministry surveillance program. **Results:** Samples collected were sorted by sex, and female samples were pooled for PCR for pathogen examination. Thirty-three (33) male samples were morphologically identified as two species with *Lutzomyia cruciata* (28, 84.8%) and *Lutzomyia deleoni* (5, 15.2%). However, molecular identification using a 658-bp fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) gene revealed previously identified *L. deleoni* to be *L. verrucarum* with a 90%–93% identity match on NCBI Basic Local Alignment Search Tool, with the species described from Peru. Phylogenetic analysis using neighbour-joining (NJ) also showed these species to cluster 100% with *L. verrucarum* that were isolated from a leishmaniasis endemic community in Peru. **Significance:** Further sample collections are planned at the geographical location where these species were collected previously to confirm the species identification. The role of exotic *L. verrucarum* on local transmission of leishmaniasis is currently unknown as this species has not been previously reported in Mexico. However, this species is actively involved in transmission in Peru, thus calling for the need of a detailed ecological study to fully understand the role of this species in the transmission dynamics of leishmaniasis in Mexico.

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Barcoding of Anurans: an Indian initiative

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Background: India is one of the major "biodiversity hotspots" for anuran diversity. A large number of species have been described from India, but it is estimated that there exists many more taxa that await discovery/identification and description. In recent years, DNA barcoding has been proposed as an efficient approach for species demarcation and cataloguing, with the emphasis on COI as a universal animal DNA barcode. Here, we initiated a study to barcode the frogs of India with the support of the Department of Biotechnology, Govt. of India. The study was undertaken in collaboration with the Wildlife Institute of India and North Odisha University. **Results:** The main aim of the study was to develop tools for DNA barcoding and to generate barcodes of anurans to ascertain species richness and endemism in amphibian assemblages in biogeographically important areas of India. We have now been able to generate barcode signatures for ~520 individuals belonging to >40 anuran species and 20 genera. For each sample, barcode signatures have been developed for at least four different mtDNA domains comprising a combined length of >2000 bp. **Significance:** To the best of our knowledge, this is probably the only and the largest barcode resource available for Indian anuran species. Our analysis suggests the presence of a number of new candidate/cryptic species pending description. Our attempts to ascertain the feasibility/suitability/efficiency of developing universal primers for anuran barcoding suggests the need of using at least two different genomic domains: one to identify the species and the other for its unique signature to be used as a DNA barcode. We also analyzed our data to ascertain the threshold limits of marker variability that may be robustly used for species demarcation. These results will be discussed during the presentation.

Species delimitation of the genus *Latrodectus* (Araneae: Theridiidae) by DNA barcode and morphological evidence

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Background: *Latrodectus* Walkenaer, 1805, the known black widows spiders, has a worldwide distribution. The genus is comprised of 31 known species. Members of this genus are important because of the toxicity of their venom to humans. However, they are taxonomically difficult to identify, due to a tortuous taxonomic history, multiple synonyms, and species revalidation, as well as a lack of discrete boundaries of morphological characters that distinguish them. Moreover, individuals of many species of *Latrodectus* present high intraspecific variation, complicating the identification caused by overlapping morphological-characters states. In addition, the arrangements and frequent use of suprageneric groups, without monophyly evaluation, generate uncertainty in the validation of several species. **Results:** Here, we hypothesized phylogenetic relationships of the genus *Latrodectus* using two gene regions, COI and alpha-latrotoxin. We also contrasted the molecular relationships with morphological variations, allowing us to establish discrete characters to identity species of *Latrodectus*. From the phylogenetic analysis, we obtained a gene tree, and the bPTP model confirms that COI is a useful marker to determine the limits among species. **Significance:** The results show a high genetic divergence among the specimens allocated under different nominal entities. In addition, the gene tree shows that suprageneric groups currently known correspond to artificial groups. Only two groups with high support were recovered and validated by robust morphological characters. Moreover, phylogeny in conjunction with the delimitation of species, approached by the bPTP model, is congruent with few but enough morphological characters that allow separating the different species. These discrete characters can be used to identify synonymous species, possible new species, and especially to clarify the relationships among the species of *Latrodectus*.

Bangladesh Barcode of Life (BdBOL)

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Background: Bangladesh covers an area of 147 570 km^2 and is home to 251 inland fishes, 402 marine fishes, 34 amphibians, 126 inland reptiles, 17 marine reptiles, 388 resident birds, 300 migratory birds, 110 inland mammals, and 3 marine mammals. It is an extraordinary situation that such great diversity still exists in an unusually overpopulated country. Unfortunately, no national database and regular monitoring system exists for these invaluable resources. **Results:** Bangladesh Barcode of Life (BdBOL) is an association that aims to establish a central coordination of activities related to DNA barcoding in Bangladesh. The BdBOL network (bdbol.net) was founded with the goal of using DNA barcoding to capture the diversity of life in Bangladesh and to use this information to monitor national biodiversity and enhance conservation strategies. Furthermore, we aim to promote the use of genetic methods in the study and monitoring of Bangladesh biodiversity through collaboration and exchange of professionals, coordination of national initiatives, creation of a network of experts in the field of DNA sequencing (scientific and practical applications), fundraising support, facilitating member's interests in the management of the genetic resources, and establishing relationships with international institutions. **Significance:** Our vision is to assemble a comprehensive library of standardized DNA barcodes as a reference resource for research and management of biodiversity in Bangladesh. Furthermore, we promote international collaboration on DNA barcoding for biodiversity conservation.

DNA barcoding of freshwater fishes of Bangladesh

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Background: The rapid increase in human population, and the subsequent intensification of agricultural, industrial, and infrastructural activities, along with deficient management, have led to the destruction of habitat, ecosystem, and biodiversity. Overfishing, the use of destructive fishing gear, and the catching of spawning and undersized fish are the main causes of loss of fish diversity and production in Bangladesh. The country has already lost more than a dozen vertebrate fauna during the last century. The lack of a proper database on the biodiversity is one of the greatest impediments for the utilization and safeguarding of our interests. The country needs to maintain the genetic identity and integrity of species in their natural habitats. **Results:** This study represents the first comprehensive molecular assessment of freshwater fishes from Bangladesh. We analysed cytochrome *c* oxidase I (COI) gene sequences for 78% of the species mentioned in the current Bangladesh Red List. Barcodes were obtained from 350 specimens, representing 195 species of freshwater fish belonging to 12 orders and 57 families. 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. **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 validates the efficiency of COI as an ideal marker for DNA barcoding of Bangladesh freshwater fish.

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DNA barcoding of freshwater fishes of Kainji Lake, Nigeria

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Background: Kainji Lake, a reservoir on the Niger River, harbors several fish species. The ichthyofaunal diversity of this lake is declining due to anthropogenic and other climatic factors. Effective management and conservation measures require accurate documentation and assessment of fish species/stocks. Multigene barcoding approaches have been used successfully to discriminate species and resolve taxonomic ambiguity. **Results:** In the present study, DNA barcodes were generated using cytochrome *c* oxidase subunit I (COI) and 16S rRNA genes for 20 species representing the following families: Mochokidae, Mormyridae, Clupeidae, Latidae, Gymnarchidae, Clarotidae, Cyprinidae, and Bagridae. The average conspecific, congeneric, and confamilial divergence values for COI were 0.3%, 16.27%, and 18.25%, respectively. COI sequences showed higher divergence values than 16S rRNA. Nucleotide diagnostic characters specific to each species were identified for COI. The neighbour-joining tree showed clustering of conspecific individuals with significant bootstrap for both genes. **Significance:** The barcodes from the present study supplement reference DNA barcodes from Kainji Lake in the Barcode of Life Data System (BOLD). The barcodes could be useful for phylogeographic studies, or to study patterns of speciation or allopatric speciation. Further, these preliminary results encourage local researchers to increase the taxon number and sampling area in order to develop a comprehensive database for Kainji Lake, Nigeria.

Comparing mini-barcoding methodology and hair-morphology to species identification of fecal samples from Neotropical felids

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Background: To circumvent problems with more-traditional and invasive methods in behavioral ecology studies of felids, molecular approaches have been employed to identify faeces found in the field. However, this method requires a complete molecular biology laboratory, and usually also requires very fresh faecal samples to avoid DNA degradation. Both conditions are normally difficult in the field. To address these difficulties, identification based on morphological characters of hairs found in faeces could be an alternative. **Results:** In this study, we tested a molecular identification method using the ATP6 region as a marker, regarded as a mini-barcoding approach, and compared its efficiency to a morphological identification key, constructed by us, for guard hairs of eight Neotropical felids: *Panthera onca*, *Leopardus tigrinus*, *Leopardus geoffroyii*, *Leopardus wiedii*, *Leopardus pardalis*, *Leopardus colocolo*, *Puma conclor*, and *Puma yagouaroundi*. For this molecular procedure, we simulated some field conditions by postponing sample-conservation procedures. Our results regarding the molecular approach were able to identify all species' samples. Part of these identifications were made from samples kept in suboptimal conditions, with some of them remaining outdoors for up to 7 days, simulating conditions in the field. A blind test of the hair morphology identification key obtained a nearly 70% overall success rate, which we considered equivalent to or better than the results of some molecular methods (probably due to DNA degradation) found in other studies. In some cases, complementary information about the known distributions of felid populations may be necessary to substantially improve the results obtained with the key. **Significance:** It appears that both techniques, hair morphology and our mini-barcoding method, can be used, depending on the available laboratory facilities and on the expected results.

Combining molecular and chemical data for species discrimination within the South African Erythroxylaceae (coca) family

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Background: The Erythroxylaceae or coca family is sub-divided into four genera: *Aneulopus*, *Erythroxylum*, *Nectaropetalum*, and *Pinacopodium*, some of which are capable of producing highly valued medicinal compounds including scopolamine, atropine, cocaine, tropacocaine, and tigloidine amongst others. Selected species within the genera *Erythroxylum* and *Nectaropatalum* are endemic to southern Africa. However, similar morphological characteristics between species within these two genera make it troublesome and often leads to unreliable identification, which can possibly be amended by chemotaxonomy and DNA barcoding. The aim of this study was to evaluate, on a chemical and molecular basis, the different characteristics of three species of *Erythroxylum* and two species of *Nectaropetalum* found in southern Africa, using an integrated approach of metabolomics and DNA barcoding analyses. Furthermore, we investigated the medicinal compound production and possible upregulation thereof in species belonging to these genera, as selected species have shown to contain tropane alkaloid "blockbuster medicines". **Results:** This study has identified discrepancies and commonalities within these genera on a chemical, as well as molecular, level. Trends were observed related to species grouping patterns, highlighting the need for a more sensitive and integrated approach to discriminate between species in these genera. Additionally, the medicinal compound upregulation in tissue culture may prove to be valuable in future medicine. **Significance:** This is possibly the first study comparing species grouping patterns based on the integration of chemotaxonomy and DNA barcoding. We report here also on our attempt to upregulate medicinal compound production in tissue cultures of selected southern African *Erythroxylum* species.

Diversity and species distributions of Glyceriformia (Annelida, Polychaeta) in shelf areas off western Africa

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Background: The present study is based on a large collection of marine benthic invertebrates sampled from shelf areas off western Africa from Morocco to Angola within the framework of the EAF-Nansen Project under the United Nations Food and Agriculture Organization (FAO). Benthic polychaetes from several hundreds grab and sledge samples have been pre-sorted to families, and presently detailed taxonomical studies are on-going. In this report, we will summarize faunistic data and COX1 DNA barcoding results for the families Glyceridae and Gonidaidae (the Glyceriformia). **Results:** Morphology-based identifications suggested a total of 20 species in our material: 9 Glyceridae and 11 Gonidaidae. Ten of these are known species from West Africa, three are new records for the region, and seven species could not be assigned to any described species. Representatives for all morphologically distinct taxa were selected for DNA barcoding. Despite a sequencing success rate of only 50% for the submitted specimens, and sequences being obtained for only 15 out of the 20 morpho-species, the successful sequences were assigned to 24 genetically different BINs (Barcode Index Numbers) in the Barcode of Life Data System (BOLD). Six of the morpho-species include two or more BINs, which indicates the presence of several more taxa in the material. **Significance:** The present study demonstrates the advantage of DNA barcoding in screening diversity in areas where the marine invertebrate fauna is poorly known, and documents a potential 70% increase in the reported species diversity of Glyceriformia in West African waters. It also highlights the need for an integrated approach of mor-

phology and barcoding if one is to achieve a profound knowledge of the regional biodiversity.

Metabarcoding plants from lake sediments: where are we and where are we going

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Background: Lake sediments are important archives of past environments, but we still know little about the actual extent to which species diversity is represented in the eDNA records. We evaluate the potential to detect plant diversity using the P6 loop region of the chloroplast *trnL* (UAA) intron from ~30 lakes from Scotland, Svalbard, Polar Urals, and northern Norway. We also develop a reference library of >1300 species using low-cover shotgun sequencing as well as a pilot study of shotgun sequencing on lake sediments. **Results:** The average potential taxonomic resolution using the P6 loop was 75%–85%. However, the amount of taxa actually detected differed among lakes. An exceptionally good record from the Polar Ural allowed inference of environmental changes over 25 000 years to a level of ecological detail rarely obtained. The dominant species were recorded in all lakes, whereas the detection of rare species is limited by (*i*) our ability to distinguish low number of reads of true positives from that of the background noise, (*ii*) taphonomic processes that may cause DNA of some rare species being more unevenly distributed in lake sediments, and (*iii*) taxonomic resolution. We were able to assemble the full plastid, mtDNA, and ITS for the majority of the species. **Significance:** The current most widely applied technique of metabarcoding plants from lake sediments is a cost efficient method with a taxonomic resolution higher than typically found with macrofossils (65%–69%) or pollen (44%–59%), but large variation exists. To increase the information gained, we suggest to (*i*) fit statistical models to improve understanding of true and false positives, allowing more certain detection of rare species, and (*ii*) increase amount and spread of sampling. Based on our pilot study, we evaluate if shotgun sequencing is increasing the taxonomic resolution and potentially also the quantitative information gained from lake sediments.

Building a comprehensive barcode reference library of the Norwegian Echinodermata through NorBOL: an ongoing effort

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Background: As part of the Norwegian Barcode of Life (NorBOL) project, the University Museum of Bergen and its collaborators mainly focus on the barcoding of marine invertebrates, including the Echinodermata. The echinoderms are considered to be a well-known group with 147 recorded species in Norway (50 Asteroidea, 40 Ophiuroidea, 33 Holothuroidea, 17 Echinoidea, and 7 Crinoidea). Many of these are common and widespread and with the exception of the crinoids, which tend to fragment very easily, most echinoderms preserve species characteristic morphological characters very well. As such, it should be feasible to build a high-quality reference library for most, if not all, known species. **Results:** So far, 475 specimens identified to 116 morpho-species have been attempted for barcoding, resulting in 381 barcodes grouping into 104 Barcode Index Numbers (BINs). The group has a high sequencing success rate (80% of specimens, 86% of species) compared with most other marine invertebrates we have worked on. We found that several samples that were initially identified as one species were allocated to multiple BINs, or to BINs containing specimens with more than one name. This may be due to unresolved taxonomy (cryptic species, discordance in name use between laboratories), misidentifications, or sequence contamination. This highlights the ever present need for careful evaluation and revisions by taxonomists once the sequencing is completed. To resolve some of these cases, barcoding of material collected at or close to type locality will be beneficial. Barcodes already in the Barcode of Life Data System (BOLD) indicate a very wide distribution for some species. **Significance:** High success rate and relatively few species make it achievable to build a reference library for Norwegian echinoderms. Limiting factors are the availability of taxonomic expertise and of suitable material. Continued sampling efforts and taxonomic work is needed.

DNA barcoding of marine macroalgae (seaweeds) of Ghana: a tool to address the need to assess and monitor the diversity of an important marine resource

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Background: In algae, apart from aiding in the identification of taxa, DNA barcoding has the possibility for applications in many other fields including biological inventory and species discovery, to quality control and forensics. DNA barcoding could have great utility with the marine macroalgae in Ghana as they are difficult to identify using morphological characters alone. Furthermore, they are an important marine resource, which holds promise for a thriving seaweed industry in Ghana (for example, agar, alginates, carrageen, and other important substances including nutraceuticals). Accurate identification is the first step in conserving and monitoring algae. An identification guide to the seaweed flora of Tropical West Africa was published in 1987 and revised in 2003. However, a considerable body of new information (recent genomic investigations) has emerged since then, resulting in taxonomic and nomenclatural changes. Therefore, an urgent revision is needed. Regional identification guides are important, but national floras are also necessary. The project began in August 2016 aiming to produce a user-friendly identification guide of the seaweeds of Ghana. A major component of the project involved DNA barcoding of the marine macroalgae occurring in Ghana. Genomic DNA was extracted from silica-dried algal material and the COI and *rbcL* gene regions sequenced using standard procedures. **Results:** A database of DNA sequences and associated data will be developed into a public resource and be available on the World Wide Web. **Significance:** The database will be useful to the staff of government agencies (particularly regulatory agencies), universities, and other organisations monitoring environmental change, studying marine diversity, assessing stocks for commercial exploitation, searching for useful chemicals, and other materials, as well as to lecturers and non-professionals with an interest in marine algae.

From genes to genomes: progress and pitfalls in barcoding the Kingdom Protista

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The protistan contribution to the diversity of life on Earth exceeds that of many, if not most, other eukaryotes combined. In addition to

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often being morphologically complex, protists possess a wealth of variability at the marker gene and genomic levels that can be exploited for "barcoding" purposes. While protistan genomics pales when compared to their bacterial and archaeal counterparts, strides in next-generation DNA sequencing are slowly closing this gap. My talk will review recent global efforts to "barcode" single-celled and multi-cellular protists using examples taken from the International Census of Marine Microbes (ICoMM), Microbial Inventory Research Across Diverse Aquatic Long Term Ecological Research Sites (MIRADA-LTERS), and the MicroB3 Ocean Sampling Day (OSD) projects. I will also highlight our recent efforts to develop barcodes for holopelagic *Sargassum*, and the work of my laboratory and others developing barcodes for members of the phylum Haptophyta. For some groups of microbial eukaryotes, barcoding can be accomplished with mere fragments of the gold standard small subunit ribosomal RNA gene, but for others it requires sequencing entire mitochondrial or chloroplast genomes. I will discuss some of the challenges encountered with cataloging protistan diversity across different biogeographic provinces, particularly in the aquatic realm. The few temporal studies performed highlight the importance of considering seasonality in sampling when performing biodiversity assessments. The Ocean Sampling Day effort has simultaneously sampled the World's ocean on the summer solstice in 2014–2015 with an eye towards repeated sampling every year. Unlike the samples collected from ICoMM, the OSD approach involves coordinated timing, standardized sampling methods, a common DNA extraction protocol, and has extended the inventory of microbial diversity to include functional potential. Leveraging existing biodiversity observatories and establishing new ones will improve our abilities to better understand the importance of microbial eukaryotes in a changing ocean and on a changing planet.

DNA barcoding and conservation of Podostemaceae in Africa

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Background: The aim of this project is to generate DNA barcodes for all Podostemaceae species in Africa; to ease species identification. The enigmatic river-weed family Podostemaceae could benefit immensely from DNA barcoding since the plants are not easy to identify using morphological characters alone. Furthermore, species in the family Podostemaceae play several ecological beneficial roles in tropical river systems. Particularly, they are important primary producers and are good indicators of river health. The survival of these plants are, however, threatened by land use practices adjoining the rivers and construction of dams on rivers across Africa. In a preliminary investigation, ~40 Podostemaceae species occurring in Africa were sampled. Total genomic DNA was extracted from silica-dried leaf material and sequenced using standard procedures. **Results:** The DNA barcode sequences of African Podostemaceae will be linked to herbarium voucher specimens, including digital images/photos, locality, literature, and uses, and will enable the accurate identification of the species. **Significance:** The data generated will be made publicly available in international genomic and DNA barcoding databases, including the Barcode of Life Data System (BOLD) and EBI/GenBank. The accurate identification of the river-weed family will be a first great step in their conversation. Conservation strategies of Podostemaceae will be discussed.

Preliminary DNA barcoding on forest birds in Yoko (Kisangani, DRC)

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Background: The richness of the Congolese forest avifauna is well known on the basis of morphological data. However, genetic data (DNA barcoding) are relatively unavailable. Some disparate sequences were obtained from museum specimens (MRAC, IRScN.Be). **Results:** This work presents sequences of the DNA-mitochondrial region of avian tissues taken from harvested birds. It forms part of a doctoral study (2008 and 2010) in Yoko, a forest reserve in the central Congolese basin located in the Kisangani region of the Democratic Republic of the Congo (DRC). The birds were captured by Japanese nets. The fresh tissues preserved in alcohol (98%) were sequenced at the Royal Belgian Institute of Natural Sciences in 2011. The methods and techniques used are as described in the user guide Nucleo®Spin Tissue. In total, 19 avian species from the Yoko forest reserve have been sequenced. **Significance:** These preliminary results show the interest of intensifying the study of DNA barcoding of the Congolese avifauna.

FENNEC — Functional exploration of natural networks and ecological communities

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Background: A central aspect of biodiversity research is to assess the species composition of complex natural communities and ecological networks. Besides traditional methods, nowadays, even communities that are hard to distinguish morphologically (like bacteria, pollen, or algae) can be reliably identified through DNA metabarcoding. For many ecological questions, the ecological properties (traits) represented in a community are more informative than just the scientific names of their members. Furthermore, other properties that are relevant for many studies, like threat status, invasiveness, or human usage, cannot be derived only from taxonomic names. Despite the fact that various public databases collect such trait information, it is still a tedious manual task to enrich existing community tables with this important functional information. **Results:** Here, we present FENNEC, a web-based workbench that eases this process by mapping publicly available trait data to the user's community tables in an automated process. Public trait information is still sparse, but what is present already helps in interpretation of community data. We applied our novel approach to a case study in pollination ecology to demonstrate the usefulness of FENNEC and also to encourage other scientists to make trait data available in public databases. **Significance:** FENNEC is a free web-based tool that aids in adding the layer of species traits to ecological community analyses. We already integrated various traits related to pollination ecology, which are readily usable for community analyses. We aim to encourage scientists to participate in trait data submission to existing trait databases and to define comprehensive, reliable, and informative trait data sets to be used by the FENNEC framework.

DNA barcoding of ornamental fishes of Jammu

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Background: Ornamental fish, also known as aquarium fish, are called living jewels. They have different colour combinations on their bodies, attractive body shapes, and numerous fin structures, which make them objects of considerable aesthetic value. Identification of ornamental fish in Jammu and Kashmir State can be challenging, especially due to the different distinct water conditions, which include both cold and warm water streams, perennial rivers, lakes, and reservoirs. There are also 250 high altitude lakes spread over an area of 40 000 ha. DNA barcoding, which uses the mitochondrial cytochrome *c* oxidase 1 (COI) gene as a target gene, is an efficient and accurate method for fish identification for assessment and conservation of dwindling diversity. **Results:** A survey of various water bodies in and around Jammu was carried out for collection of different fish species available. Fish were caught from slow-moving streams and ponds with the help of drag nets

and cast nets, and preserved in 10% formalin and 100% alcohol. Identification of collected ornamental fish was done using traditional taxonomic methods. DNA extraction, PCR amplification with species-specific PCR primers, and DNA sequencing will be carried out, which will offer great potential for the identification of species. **Significance:** A first attempt has been made to create a database of DNA barcodes that will be helpful in identification of fishes and also to authenticate the diversity of ornamental fish present in Jammu areas. This study will prove a milestone in advanced research by using modern techniques and easy to handle methods for fish identification in different geographical limits. It will also address some taxonomic issues that need further investigation.

Eukaryotic diversity in the largest glacial lake of Iberian Peninsula: a metabarcoding approach

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Background: Biodiversity assessments of different environments are important if we want to understand and face the persistent loss of diversity. They are also needed to have a more realistic view of the tree of life. Metabarcoding analyses by high-throughput sequencing provide a valuable tool for describing the community composition and offer several advantages over traditional morphological surveys, such as obtaining a large amount of data in a relatively short period of time. Most aquatic surveys of biodiversity have so far focused on marine environments, with few analyses done in freshwater environments, even though they may harbour novel eukaryotic diversity. We performed a metabarcoding study in Sanabria Lake, the largest glacial lake of the Iberian Peninsula, which is currently under an eutrophication process. Our objectives were to survey the general eukaryotic diversity, assess the community structure and search for novel clades within Opisthokonta, the clade which contains animals, fungi, and several unicellular lineages. **Results:** We collected water from five different sampling sites at different depths. We used three filter sizes (20, 5, 0.8 μm) for the water column. Sediments and biofilms were also collected. We sequenced the V4 region of the 18S rRNA gene for each sample using the Illumina Mi-Seq platform. We processed our data using the Obitools pipeline and R. **Significance:** This work will provide a perspective on the community structure of a large lake and an insight into the freshwater eukaryotic diversity. Observing new clades among opisthokonts will help to elucidate the origin and evolution of the two multicellular groups within opisthokonts: animals and fungi. Moreover, it will contribute to generate a more realistic tree of life. Finally, our work may be a preliminary phase in biomonitoring for future conservation actions.

Global metabarcoding survey from TaraOceans expedition uncovers novel diversity in Opisthokonta

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Background: Opisthokonta is a clade of eukaryotes, which contains animals, fungi, and several unicellular lineages. These unicellular opisthokonts are essential to address evolutionary questions, such as the origins of animals or fungi. Moreover, they are ecologically relevant because of their variety of forms (parasites, symbionts, and free-living). Finally, they are necessary to fully describe the eukaryotic community of a habitat. However, the diversity of these unicellular opisthokont lineages remains poorly described. Metabarcoding is a crucial tool to overcome this problem for two reasons. First, large amounts of data are sequenced, so there is a high possibility of finding hidden diversity. Second, the data is DNA, which means a faster identification of the organisms. **Results:** To fill this gap in opisthokonts, we analysed 18S rDNA metabarcoding data from the TaraOceans expedition, which covers 1086 marine samples over the world. The complete dataset (6 294 617 barcodes) was examined with two goals. First, we looked for molecular novelties within Opisthokonta using network analyses based on the graph theory of the putative novel operational taxonomic units (OTUs). Phylogenetic placements and phylogenies were later performed to test these novelties. Second, we obtained ecological patterns across oceans, depths, and size fractions. We also calculated ecological parameters, such as alpha and beta diversity, and performed community analysis. Our preliminary results uncover hidden diversity both within and between some unicellular opisthokont lineages. We also describe for the first time the marine ecology of Opisthokonta from a global perspective. **Significance:** Our research provides a framework for future studies to assess molecular novelty using graphical networks. Moreover, the ecological inferences together with the phylogenies of the novel clades will not only help to gain insights about the nature of the unicellular relatives of animals and fungi but will also help to draw a more realistic tree of life.

Development and international validation trial of a comprehensive, multi-locus DNA metabarcoding method to identify endangered species in complex samples

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Background: DNA metabarcoding holds great promise for species identification in complex samples such as food supplements and traditional medicines (TMs). Such a method would aid CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) enforcement officers to combat wildlife crime by preventing illegal trade of endangered plant and animal species. The objective of this research was to develop a multi-locus DNA metabarcoding method for wildlife forensic species identification and to evaluate the applicability and reproducibility of this approach across different laboratories. **Results:** We developed a DNA metabarcoding method utilizing 12 DNA barcode markers with universal applicability across a wide range of plant and animal taxa and suitable for identification of samples with degraded DNA. A newly developed bioinformatics pipeline with user-friendly web interface was used to analyze Ilumina MiSeq data for 15 well-defined experimental mixtures. The performance of the DNA metabarcoding method was assessed in an international validation trial by 16 laboratories, in which the method was found to be highly reproducible and sensitive enough to identify species present in a mixture at 1% dry weight content. **Significance:** The advanced, multi-locus DNA metabarcoding method assessed in this study provides reliable and detailed data on the composition of complex food products, including information on the presence of CITES-listed species. The method provides improved resolution for species identification, while verifying species with multiple DNA barcodes contributes to an enhanced quality assurance.

The spiders of Pakistan: commencing the assembly of a national DNA barcode reference library

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Background: The broad adoption of DNA barcoding is accelerating the documentation of animal diversity. While DNA barcode coverage

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for spiders is well advanced in some regions, current records only provide coverage for about 12% of the 46 000 species known in this order. In total, records are available for 100 000 spiders, most of which have been assigned to a Barcode Index Number (BIN), a strong proxy for species. However, there has been no DNA barcode coverage for the spider fauna of Pakistan. **Results:** Nearly 1800 spiders from sites across Pakistan were identified morphologically before being sequenced for the barcode region of COI. The resultant sequences were assigned to BINs, and species were discriminated by neighbour-joining trees and barcode gap analysis. Morphological study placed the specimens in 28 families. Most (1579) of the specimens could be assigned to 113 named species, but the rest (217) could only be placed to genus or family and an interim species (87). In total, 1796 sequences were assigned to 218 BINs. The 113 named species were allocated to 127 BINs as 10 species showed BIN splits, while the 87 interim species were assigned to 88 BINs with two showing BIN split while one lacked a BIN assignment. Maximum conspecific divergence ranged from 0% to 5.3%, while congeneric distances ranged from 2.8% to 23.0%. With the exception of one species pair, the maximum intraspecific distance was less than the nearest-neighbour (NN) distance. Only one fourth of the BINs detected in this study were known from other countries. **Significance:** The study initiates the construction of a barcode reference library for the spiders of Pakistan. BIN splits and high intraspecific divergence in some known species suggest the presence of cryptic species complexes. The low level of BIN overlap with other regions highlights the importance of constructing regional DNA barcode reference libraries.

Barcoding Norwegian water bears (*Tardigrada*)

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Background: Tardigrades are microscopic animals inhabiting the most ecosystems throughout the world. They even live in thin films of water surrounding various substrates in terrestrial habitats. DNA barcoding of tardigrades can be challenging because (*i*) species identification often requires slide mounting and magnification up to 1000×, (*ii*) there are several cryptic and semi-cryptic species complexes, (*iii*) eggs and multiple specimens from the same population are often needed for correct species identification, and (*iv*) recapturing voucher specimens after DNA extraction is difficult. **Results:** We developed a methodological pipeline with preliminary identification of live animals to genus level in a compound microscope before DNA extraction of single specimens. After extraction in a one-step solution, the cuticles and the sclerified structures of the feeding apparatus were retrieved and slide mounted in Hoyeras solution as vouchers. In a preliminary test, 84 extracts were shipped to the Canadian Centre for DNA Barcoding (CCDB) in Guelph for single pass barcoding through the NorBOL agreement. Sixty-two specimens (74%) received sequences, of which six were contaminants and 25 did not meet the formal barcode requirement due to sequence quality. The actual vouchers of 48% of the specimens were lost in the extraction process. Nevertheless, the test resulted in 18 BINs in the Barcode of Life Data System (BOLD) and seven named species. In addition, some unnamed BINs might constitute species new to science. **Significance:** Although time-consuming, individual DNA barcoding of tardigrades can contribute significantly to an important part of the reference library. An approach where voucher cuticles and paragenophores from the same population are preserved is crucial, as is the barcoding of multiple specimens of the same species to buffer failed sequencing and lost vouchers. We use this approach in the recently funded project "Tardigrades in Norwegian Forests" that aims to map tardigrade species associated with different forest types and habitats in Norway.

Towards a DNA barcode reference database for spiders and harvestmen of Germany

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As part of the German Barcode of Life campaign, over 3500 arachnid specimens have been collected and analyzed, which include ~3300 Araneae and 200 Opiliones, representing almost 600 species (median: 4 individuals/species). This covers about 60% of the spider fauna and more than 70% of the harvestman fauna recorded in Germany. An overwhelming majority of the species could be readily identified through DNA barcoding. The median barcode distances between the nearest-neighbour species were around 9% in spiders and 13% in harvestmen, while in 95% of the cases, intraspecific distances were below 2.5% (median=0.3%) and 8% (median=0.2%), respectively. However, almost 20 spider species, most notably in the family Lycosidae, could not be separated through DNA barcoding (although many of them present discrete morphological differences). Conspicuously high intraspecific distances were found in even more cases, hinting at the presence of cryptic species in some instances. A simple new program, DiStats, was developed, which calculates the statistics needed to meet DNA barcode release criteria. Furthermore, new generic COI primers, useful for a wide range of taxa (in addition to arachnids), were introduced.

Opening up collections of barcoded samples through the Global Genome Biodiversity Network

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DNA barcoding is "big science" and a very widely used tool for species identification and discovery. Thanks to barcoding projects, millions of tissue and DNA samples, from hundreds of thousands of species are being accumulated in collections around the world. These samples represent a highly valuable resource for biodiversity research due to the high quality of underlying species determinations, associated morphological vouchers, digital images, and overall broad taxonomic sampling. Making these collections visible and accessible is important for molecular biodiversity research and will ensure an increased impact of barcoding projects. With the Barcode of Life Data System (BOLD), we have a sequence database (and analysis tools) for sharing all barcode data produced worldwide. The Global Genome Biodiversity Network (GGBN) portal (http://data.ggbn.org) can easily be linked to BOLD and offers a free and transparent platform to merge contents of distributed collection databases into a single access point, bridging the gap between biodiversity repositories, sequence databases, and research results. Barcoding samples are not the only source that can contribute to this end, but a very valuable one. GGBN is also a lively international network of biodiversity institutions sharing an interest in long-term preservation of molecular samples. Collaborative activities are aimed at ensuring consistent quality standards for DNA and

tissue collections, at improving best practices for the preservation and use of such collections and at harmonizing exchange and use of material in accordance with applicable legislation and conventions.

In-silico assessment of five chloroplast intergenic regions in the family Poaceae for DNA barcoding

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Background: DNA barcoding was proposed to facilitate systematic species identification, biodiversity monitoring, and conservation. Unlike animals, the application of DNA barcoding is more complicated in plants because of the larger number of regions that need to be sequenced, compared to the single mitochondrial COX gene used in animals. After identifying the DNA barcode as a key method in the UN strategic plan for biodiversity in Cancun 2016, the scientific community was in urgent need to define and evaluate standard DNA barcoding loci in plants that could be utilized at a broad scale. Here, we assessed five intergenic regions proposed in our previous study. We retrieved the available 147 Poaceae chloroplast genomes in GenBank chloroplast organelle database. Then, we designed primers and extracted five chloroplast intergenic regions (*trnFM-trnT*, *trnD-psbM*, *petN-trnC*, *matK-rps16*, and *rbcL-psaI*) for each of the downloaded chloroplast genomes using python script. CLC software was used to perform multiple sequence alignment (MSA) and NJ phylogenetic trees for the five regions in 147 genomes to assess its ability to discriminate different species within the family. **Results:** The region *petN-trnC* extracted length ranged between 400 and 1000 bp, *rbcL-psaI* region length ranged between 800 and 2145 bp, *matK-rps16* region length ranged between 1230 and 1515 bp, *trnD-psbM* region length ranged between 575 and 1250 bp, and *trnFM-trnT* region length ranged between 960 and 2675 bp. Our results showed outstanding species discrimination power for the five regions, constituting 94.55%, 93.19%, 89.11%, 87.07%, and 86.39% for *trnFM-trnT*, *rbcL-psaI*, *matK-rps16*, *petN-trnC*, and *trnD-psbM*, respectively. **Significance:** We recommend using any of these regions individually or in combination, if necessary, as a DNA barcode in Poaceae. Also, we encourage further investigations on using these regions within other plant families.

Intraseasonal variation in species richness and abundance of ectomycorrhizal fungi as influenced by microclimate in the forest reserve of Ouémé Supérieur in northern Benin

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Background: This study aims at assessing the variation in species richness and abundance of ectomycorrhizal fungi. Nine permanent plots of 2500 m² were installed in three different phytocenoses, dominated each by *Isoberlinia doka* (V1), *Isoberlinia tomentosa* (V2), and *Uapaca togoensis* (V3). Mycological surveys were conducted at a frequency of 2 times/place/week during 17 weeks. To record microclimatic parameters, one datalogger was installed in the centre of each plot and calibrated to record air, soil humidity, and temperature for 30 min. We recorded, among others, the presence/absence of fungal species, the number of fruiting bodies, and the fresh biomass per plot. **Results:** The study reveals a significant variability of air and soil temperature and humidity between vegetation types (p=0.2; F=11, 2 and p=0.01, F=11, 16) ranging from 26.9 to 30 °C (all plots and vegetation types) and from 40% to 90%. Six (6) homogeneous fruiting phases were detected with the highest species richness (15 species/ha) and abundance (500 fruit bodies/ha all species) recorded during August. Species richness and abundance of mushrooms were not positively correlated with air and soil temperatures. It was positively correlated with the relative humidity and soil water content (p=0.049, r^2=21.2; p=0.033, r^2=17.5, respectively). The intense fructification phase of edible fungi is preceded by a sudden drop in air and soil temperature (from 30 to 26.9 °C and from 32 to 25.6 °C, respectively) and a rapid increase of air humidity (from 40% to 90%) and soil water count (from 0.07 to 0.16 m³/m³).

Developing a DNA barcode scanner for conservation

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Transnational environmental crime has become an exponential driver of species extinction in ecosystems across the world. In response to growing wealth in emerging economies, the wildlife and illegal timber trade market now measures in the billions of dollars and threatens the survival of iconic species. This black market is disrupting natural communities, and depleting innumerable species, some to the brink of extinction. Timber and wildlife product sources are difficult to identify, particularly when turned into products such as furniture, filets, powders, butchered meat, or oils. Although DNA analyses can determine whether a seized product is derived from an illegal species, these technologies are absent where they are most needed in the field. Our DNA Barcode Scanner Project is a collaborative effort between Conservation X Labs, Smithsonian Institution, Consortium for the Barcode of Life, WWF, Oceana, University of Washington, and others, with the goal of creating a handheld POC device that utilizes barcode sequences in animal and plant genomes. Our project is bringing together a diverse team of engineers, geneticists, and conservationists whose goal is not to make a 100% clinically accurate device, but to engineer a decision support tool: a low cost, simple to use, robust, highly modular molecular sensing device that allows citizens or officials to rapidly determine whether to investigate a timber or wildlife shipment more deeply or a corporate seafood buyer to detect problems in their supply chain. We need to develop a product that supports decision making and traceability in the environments where they matter in the field, within the developing world, with the least number of steps possible, at lowest cost, with the highest resilience, and lowest complexity.

Environmental DNA reveals tropical shark diversity and abundance in contrasting levels of anthropogenic impact

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Background: Sharks are key components of virtually all marine trophic webs, but many species are suffering from overexploitation and stock declines. The conservation of sharks and their functions in an ecosystem and the development of management strategies rely heavily on our ability to assess and monitor their distribution and abundance. However, the assessment of mobile species in marine environments remains challenging, often invasive, resource-intensive, and dependent on taxonomic expertise. The advent of parallel sequencing technologies offers new, powerful tools for biodiversity assessment. This includes the retrieval, amplification, and sequencing of fragments of environmental DNA (eDNA) shed by organisms in aquatic habitats, with the possibility to rapidly gauge vast amounts of information on taxonomy and community structure. **Results:** Here, we employ this

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novel, rapid, and non-invasive eDNA metabarcoding approach, specifically targeted to infer shark presence, diversity, and abundance across a range of impacted versus protected/remote areas in both tropical Pacific and Atlantic regions. We detect tens of shark species whose geographical distribution and relative abundance coincide with established knowledge on biogeographic patterns and levels of anthropogenic pressure and conservation effort. These findings indicate that eDNA metabarcoding can be effectively employed to study shark diversity in pelagic habitats. **Significance:** Further developments in this field have the potential to drastically enhance our ability to assess and monitor elusive oceanic predators such as sharks, which are particularly difficult to quantify by means of traditional methods, and lead to improved conservation strategies.

High species-level diversity found for Collembola in the Namib Desert

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Background: The diversity of belowground invertebrates including springtails (Collembola) is relatively unknown in the Namib Desert. Previous morphological studies have found only four species on the basis of traditional taxonomy. Here, we undertook further sampling of Collembola using flotation of soil samples collected around the vicinity of Gobabeb in the central Namib Desert. Over 400 individuals were collected from 20 sites and analysed for sequence diversity at the COI gene locus. All sequences were entered into the Barcode of Life Data System (BOLD) and assessed for diversity using the Barcode Index Number (BIN) algorithm. **Results:** In total, 434 individual springtails were recovered from 77 soil samples and 341 COI sequences >618 nucleotides were obtained (79%). Sequences did not closely match with any record previously available on BOLD (<85% similarity in all cases). Using the BIN algorithm, we found a total of 43 BINs with a range of 1–14 BINs per site. Only nine BINs were found at more than one site, suggesting limited dispersal among sites. **Significance:** The Namib Desert has much higher levels of diversity among populations of Collembola than previously known. We speculate that reasons for this diversity may include (*i*) the age of the habitat (>180 MY), (*ii*) physical and chemical patchiness (e.g., moisture, geology), and (*iii*) limited dispersal.

Spatial and temporal variation of macroinvertebrate eDNA in Dutch freshwater lakes

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Background: In order to implement environmental DNA (eDNA) monitoring into Dutch freshwater quality management we seek to validate metabarcoding of eDNA. This technique can be used for non-invasive sampling of freshwater and can be more easily standardized across monitoring programs. eDNA methods have already proven their use in the monitoring of specific organism groups, such as fish or invasive species. However, to start incorporating these molecular tools into the monitoring of groups such as macroinvertebrates, more understanding is needed of how eDNA reflects local communities. We have looked at the detection of macroinvertebrates in eDNA samples, with the ultimate aim of fast and more reliable species-level identifications for freshwater monitoring programs in the European Water Framework Directive. **Results:** We have studied the changes of eDNA patterns over time, and compared eDNA metabarcoding results with morphological surveys of macroinvertebrates. eDNA samples show variation in community composition throughout the seasons. Analyzing the patterns show optimal sampling moments for various taxa. Spatial sampling and replications in sampling, extraction, and amplifications show that replicates are a necessity and that eDNA is not homogeneous. **Significance:** The observed variations in "eDNA communities" will bring a better understanding of how eDNA reflects the traditionally observed communities. Coupled with the use of replicates in the right steps of the eDNA analysis, this will lead to better strategies for sampling and processing eDNA samples for the monitoring of freshwater quality. Whilst molecular species lists are currently not an exact match with morphological ones, the additional taxa obtained by sequencing (now considered mere "bycatch") harbor great potential for incorporation into monitoring programs. They also provide insight into the gaps in current databases and knowledge of freshwater life. Metabarcoding of eDNA will offer us a uniform and cost effective monitoring tool for freshwater nature conservation policy purposes.

Metabarcoding of chironomids 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 poorly understood. The dipteran insect family Chironomidae, informally known as non-biting midges, is a highly diverse taxon with more than 10 000 described species globally and the most abundant insect group in many streams. However, they often only play a minor or undifferentiated role in ecological studies, as well as stream ecosystem assessments, due to the difficulties associated with their identification, in particular when based on larval morphology. DNA metabarcoding offers a promising approach to more accurately capture this species diversity. Here, we used DNA metabarcoding to obtain and evaluate chironomid 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 59 325 chironomids have been sampled from two microhabitats, substratum and leaf litter packs, from 64 mesocosms each (8 replicates per treatment). On family level, chironomids of the substratum responded positively to added fine sediment and flow velocity alteration and chironomids of leaf litter packs negatively to altered flow velocity. Using operational taxonomic units (OTUs) obtained through DNA metabarcoding the individual response patterns could further be disentangled. **Significance:** Morphological identifications can be insufficient when dealing with morphologically "difficult" groups such as chironomids. When studied only at higher taxonomic levels, response patterns of species to environmental variables are masked, potentially leading to incorrect conclusions. Here, metabarcoding provides the relevant resolution and thus facilitates the investigation of multiple stressor effects on individual species. The combination of manipulative field studies together with metabarcoding furthermore holds great potential for targeted assignment of ecological traits to OTUs, which in return makes these available for water quality assessments.

Status of DNA barcode reference libraries for New Zealand freshwater and terrestrial invertebrates

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The dynamic geological history of New Zealand, coupled with its long-term isolation from Australia (~65 mya), has resulted in diverse and largely endemic invertebrate fauna. This study aimed to evaluate the availability of DNA (COI) barcode records for New Zealand's freshwater and terrestrial invertebrates and to highlight gaps in these data. We summarized all current public records on the Barcode of Life Data System (BOLD) housed in a dataset (DS-NZINVR). The dataset currently

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contains 26 852 New Zealand freshwater and terrestrial invertebrates representing 5 phyla, 50 orders, 302 families, 652 genera, and 1056 species. There are also 2886 Barcode Index Numbers (BINs) thus exceeding the number of recognized species two-fold. Examples of comparatively well-covered groups include zooplankton (*n*=77 species, 134 BINs), freshwater insects (*n*=307 species, 349 BINs), and spiders (*n*=81 species, 206 BINs). In contrast, New Zealand Acari (mites) have public records for only three species while the entire annelid phylum is limited to five sequences (*n*=3 species). These data will be used to focus future barcoding efforts and to highlight groups that have been adequately covered and could be immediately used for conservation, biosecurity, and ecological studies/applications.

Using pollen DNA metabarcoding to construct quantitative pollinator networks

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Background: To study pollinator networks in a changing environment, we need accurate, high-throughput methods. Previous studies have shown that relative to field observations, more highly resolved networks can be constructed by studying pollen loads taken from bees. DNA metabarcoding potentially allows for faster and finer-scale taxonomic resolution of pollen, compared to traditional approaches, e.g., light microscopy, but has only recently been applied to pollination networks. I will review data from the literature and ongoing work using pollen DNA metabarcoding to construct quantitative pollinator networks, using pollen sampled from bees. **Successes and caveats**: Using a next-generation sequencing approach and comparison to comprehensive reference libraries has enabled species-level identifications for complex mixtures of pollen species. Typically, identifications are at least to genus level, in comparison to microscopic identification, which is often only to family level. Identifications are to some extent limited by absence of species in reference databases, leading to different identifications with different markers. There have been mixed results regarding whether or not the pollen DNA metabarcoding method is quantitative. However, quantitative pollination networks can be built, based on frequencies of interactions, and used to better understand the plant–pollinator interactions in the ecosystem. **Significance:** Pollen DNA metabarcoding has provided a significant advancement in methodology for the construction of pollinator networks, and there have been substantial method developments since the last iBOL meeting in 2015. As methods for DNA metabarcoding improve over the next few years, I expect that we will gain further advantages in efficiency and resolution over microscopic identification of pollen, opening further opportunities not just in plant–pollinator interactions, but across diverse fields of research.

Quantitative assessment of DNA metabarcoding with constructed species mixtures

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Background: Pollen DNA metabarcoding—marker-based genetic identification of mixed-species pollen samples—has applications across a variety of fields in biological research. Recent studies have demonstrated proof-of-concept of species-level pollen identification using standard DNA barcode markers. However, there have been few studies testing the robustness of these methods, or testing the quantitative matching of pollen grain counts to sequence reads using constructed samples of known composition. We tested the ability of standard pollen DNA metabarcoding methods using the Illumina MiSeq platform with the markers *rbcL* and ITS2 to identify and quantify species in artificial mixtures of varying species richness (1–9 species), taxonomic relatedness (within genera to across class), and rarity (5%–100% of grains in a sample). We also examined the rate of false positives, and considered whether these were the result of misidentification, sequencing error, or contamination. **Results:** Species composition determinations were largely correct, with rarity of grains in a sample driving the likelihood of their detection. Sample species richness and taxonomic relatedness of species in a sample, however, did not strongly impact correct determinations. More species-level identifications were obtained with ITS2 than with *rbcL*. False positives were usually the result of either the correct species not being represented in the reference database or very closely related species having identical barcode sequences. **Significance:** Our results show that DNA metabarcoding is not quantitative. The proportion of reads for each species was only weakly correlated with its relative abundance. This may be the result of copy number, DNA isolation, or amplification bias. Further research into biases will enable quantification of species proportions in addition to information on species presence, which could be groundbreaking in many applications of pollen DNA metabarcoding.

The Cape gum bushes: taxonomy and diversification of *Pteronia* (Asteraceae)

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Background: *Pteronia* L. is a large, often aromatic, shrubby genus comprising ~70 species, most of which favour arid habitats within the Greater Cape Floristic Region. The genus was last treated by Hutchinson and Phillips in 1917, who recognised four sections based exclusively on leaf indumentum. However, this classification is considered largely artificial and in need of reassessment. **Results:** A systematic study was carried out to investigate the phylogenetic relationships of the southern African *Pteronia* using molecular (DNA sequences), morphological, and anatomical data as well as to infer biogeographic patterns and estimate the divergence times using a relaxed clock dating analysis in BEAST. Phylogenetic analyses of the genus were based on two nuclear (internal and external transcribed spacer: ITS, ETS) and one plastid (*trnL-F*) DNA sequence data for 84 samples representing 70 taxa. Our phylogeny revealed that *Pteronia* is monophyletic with four main clades recovered and that the current infrageneric classification is unnatural. Although none of the groupings corresponds to the previous infrageneric classification, they can all largely be identified by morphological characters. A comprehensive taxonomic revision of *Pteronia* was also completed in which we recognised 75 species, of which three are new. **Significance:** *Pteronia* has been identified as one of the priority genera for taxonomic research in South Africa. This study has therefore provided a detailed taxonomic treatment of the genus a century after it was last reported by Hutchinson and Philips in 1917. Also, hypotheses of species relationships and evolutionary history of the genus have been established as bases for further studies.

Biogeography of southern African legumes

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Background: Legumes are an important component of the southern African flora, comprising nearly 1600 of the region's 2300 angiosperm

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species. Although legumes are present in all major biomes of the region, various biomes comprise distinct legume assemblages partitioned into the major biomes (fynbos, grassland, savannah). For instance, the fynbos biome (about 760 legume species) is almost exclusively occupied by papilionoid legumes, belonging mostly to the tribes Crotalarieae, Indigofereae, Podalyrieae, and Psoraleeae. Conversely, the mimosoid legumes that characterize the savannah are nearly absent in the fynbos and grasslands. Drivers of these biogeographic patterns and the high legume species richness are not known. Using a dated phylogeny based on DNA barcode markers (*matK* and *rbcLa*), from public databases and those from our own collections, this study sought to determine the timing, frequency, and direction of legume dispersal and speciation events among the southern African biomes and whether particular lineages exhibit differential diversification rates within and between biomes. Phylogenetic relationships and lineage divergence date estimates (based on secondary calibrations) were determined using BEAST. **Results:** Phylogenetic relationships among the southern African legumes were consistent with recently established intrafamilial relationships of legumes, globally. Ancestral trait reconstructions revealed evidence of multiple independent dispersal events from the grassland/savannah biomes into the fynbos biome, followed by rapid radiation of the major lineages in mid- to late Miocene. Few incidences of dispersal out of the fynbos biome were also observed. Possible drivers of the diversification patterns are discussed. **Significance:** These DNA barcode data help provide an improved understanding on the divergence and diversification of legumes across the various biomes in southern Africa. The information provided here is essential especially in setting the conservation priorities for legumes and other related plant groups.

Assessing the impacts of land use change and seasonality on arthropod communities in the northern edge of the tropics

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Background: Changes in the abundance, diversity, and composition of arthropods are linked to human-induced habitat modification including climate, deforestation, agriculture, and urbanization. These biodiversity changes alter ecosystem processes and influence ecosystem resilience to environmental perturbations. Land-use change may influence biodiversity by decreasing available habitats, food resources, and altering biotic interactions. Arthropods play key roles in the ecosystem and in global biogeochemical processes. Despite their ecological importance, knowledge on seasonal dynamics and community response to land-use change is limited due to their small body size and identification challenges. We determined whether arthropod community structure varied between habitats (forest and rubber) and seasons (wet and dry) and whether different functional groups displayed habitat and seasonal preferences. Litter arthropods were collected monthly for 12 months from 10 forest-rubber matched sites (120 samples). Genomic DNA was extracted from each bulk sample, and the COI gene was targeted for amplification using an Arthropoda-specific primer and a metabarcoding protocol. **Results:** We obtained 3084 operational taxonomic units (OTUs), from >2 000 000 Illumina Miseq reads, after rigorous bioinformatic filtering and clustering at 97% similarity. OTU richness and community composition exhibited significant habitat differentiation but little or no seasonal variation. As expected, richness was higher in forests than in rubber and lower in dry than in wet season. However, the richness of some groups, such as Chilopoda, Collembola, Hymenoptera, Isoptera, and Orthoptera were not affected by land use, whereas the richness of Collembola and Orthoptera did not respond to seasonality. **Significance:** This study provides new, in-depth knowledge on seasonal changes in diversity and community dynamics of arthropods in the northern edge of the tropics, where unprecedented land-use change is causing biodiversity loss and loss of vital ecosystem services. As the effects of anthropogenic activities are projected to become progressively more severe in the future, urgent solutions are needed to protect remnant biodiversity in Xishuangbanna, China.

Towards a phylogeny in the *Schizoglossum* complex and its allies (Apocynaceae: Asclepiadoideae)

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Background: The *Schizoglossum* complex is placed in the subfamily Asclepiadoideae (Apocynaceae). The eastern and southern African centre is one of the most diverse with ±86 genera. The southern African subtribe Asclepiadinae is the most diverse, representing ±64% of the taxa, and the group under study is placed here. The circumscription of this generic cluster has always been unsatisfactory and consists of many closely related genera. Despite existing keys to discriminate between the genera, there are several morphological characters that intergrade between them. Historically, the flower and corona structures were important for delimitation of genera and species, but recent studies have shown that many of these characters are not homologous due to convergent evolution rather than common ancestry. This, coupled with relatively recent radiation and interbreeding, may be causal reasons for the conflicting generic taxonomies within the subtribe. **Results:** Phylogenetic analyses based on the two barcoding regions (*rbcLa* and *matK*) and the nuclear region ITS, together with a systematic study including morphological examination of specimens, are presented. From the resulting phylogeny three well-supported clades were derived. A polyphyletic clade of mainly *Aspidoglossum* is the largest and included both the *Schizoglossum bidens* complex and the genus *Miraglossum*. In order to achieve monophyly, these taxa must be subsumed within the current genus *Aspidoglossum*. *Schizoglossum* and *Stenostelma* are the other two well-supported genera. The splitting of *Aspidonepsis*, recognition of *Schizoglossum montanum* as a separate genus, and re-instatement of *Lagarinthus* to accommodate *Schizoglossum aschersonianum* and *S. linifolium* are proposed. The presentation highlights some of the outcomes of the revision based on the morphological study and phylogenetic relationships of a selection of genera from the subtribe Asclepiadinae in Africa. **Significance:** The outcome of this study is a first step towards understanding the non-biased generic circumscription of a large number of African representatives for Apocynaceae in Africa.

Managing the freshwater native and non-native flora of South Africa using DNA barcoding

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Background: Freshwater ecosystems and their associated biodiversity are under increasing threats from anthropogenic activities, regardless of their importance to human well-being. As a result, global commitments by most governmental and non-governmental agencies to mitigate these threats to freshwater flora diversity are insufficient. This is due in part to (*i*) challenges inherent to species identification, (*ii*) difficulties in assigning a good genetic marker for species identification, (*iii*) phylogenetic complexities within major groupings, and (*iv*) the lack of a centralized and open access database for freshwater plant species, impeding the appraisal of biodiversity changes over time. Here, we surveyed the freshwater biodiversity of South Africa and used the core DNA barcoding

markers (i.e., *rbcLa* and *matK*) together with an additional non-coding marker (*psbA-trnH*) in an attempt to provide solutions to the challenges listed above. **Results:** We observed that, of the ~1500 freshwater native and non-native plants present in South Africa, only a marginal number (<7%) of them have existing DNA barcoding data. Additionally, using the three different markers, we observed that the noncoding *psbA-trnH* spacer was the most reliable marker for identifying freshwater plants in South Africa. Moreover, we identified some alien plant species, particularly the invasive and prohibited species commonly traded in major aquaria in South Africa. **Significance:** We discuss the potential this data set could have in answering research hypotheses related to freshwater flora and the implications for the management of freshwater biodiversity in South Africa.

Identifying New Zealand's spiders using DNA barcoding

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Morphological identification of spiders is often complicated by phenotypic plasticity and sexual dimorphism. New Zealand has a highly endemic spider fauna consisting of an estimated 3000 species. However, less than half of these have been formally described. Given such a high diversity, it is critical to seek interim measures of species diversity and identification methods for undescribed taxa. This study aimed to determine whether New Zealand's commonly observed spiders could be successfully delineated using COI DNA barcodes. Our analysis consisted of a 550-bp region of the COI gene taken from 94 recognized species (*n*=575 COI sequences). A further 198 sequences belonging to 40 undescribed morphospecies were also included. COI sequences reliably placed specimens into the corresponding molecular operational taxonomic units (OTUs) associated with individual species, regardless of sexual dimorphism or morphological plasticity. This inventory provides the foundation for a COI library for New Zealand's most commonly encountered spiders and provides a means of identifying endemic and introduced species.

Scaling up the generation of reference quality genomes across a range of vertebrate diversity

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At the Wellcome Trust Sanger Institute we are working on scaling up sequencing and assembly of vertebrates at reference quality, to support the Vertebrate Genomes Project (VGP) in association with the Genome 10K Project. The project, which commenced in 2016, is currently targeting 50–100 species from three main groups of vertebrates, including several fish groups, the caecilian amphibians, and various species of rodents. Currently, the main weight is placed on sequencing fish groups, more specifically members of the Notothenioinidae (Antarctic fish), members of the family Cichlidae related to the Haplochromine radiation, various strains of zebrafish (*Danio rerio*) and other closely related Cyprinidae, and species of the ananbantoid group (gourami) of fishes. Furthermore, through the VGP we are evaluating a range of sequencing technologies including PacBio, Oxford Nanopore, 10X Genomics, BioNano, and Illumina for generating reference genome quality data. Ultimately, we are aiming to achieve reference quality genomes with greater than 1 Mb contig N50, 10 Mb scaffold N50, and 90% DNA assignment to chromosomes. Novel contig scaffolding approaches are also being explored, such as using linkage disequilibrium from population variation data to order and orient contigs. Through an ongoing collaboration with the European Bioinformatics Institute (EBI) we are also striving to update data deposition in relevant archives, ensuring efficient gene annotation and presentation in Ensembl. We will present preliminary results from the cichlid fish *Astatotilapia calliptera*, several strains of zebrafish *Danio rerio* and the grasshopper mouse *Onychomys torridus*. Within 2017 we are aiming to scale up sequencing efforts to provide genome data for additional species, including *Gouania willdenowi* (Blunt-snouted clingfish), *Erpetoichthys calabaricus* (reedfish), *Mastacembelus armatus* (tire track eel), and *Acomys russatus* (golden spiny mouse). This initiative will provide a valuable resource of genome data to the community, to be used for more in-depth investigations of evolutionary relationships of vertebrates.

A DNA barcode reference library and ecoregion analysis of Nearctic wolf spiders (Araneae: Lycosidae)

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Background: Species diversity on our planet follows complex patterns governed by historical, physical, and biological parameters, resulting in the classification of well-defined ecoregions usually dominated by specific plant communities. As spiders are predators, they are not directly dependent on plants for nutrition but may have similar distributional patterns. Until now, comprehensive analysis of these patterns has been prohibited by arduous specimen identification and a high incidence of cryptic species. However, DNA barcoding can circumnavigate many of these barriers by delineating species and accelerating specimen identification. We utilize this method to build a DNA barcode reference library for Nearctic Wolf spiders (Lycosidae), one of the four largest spider families in North America, and investigate distribution patterns across the Nearctic ecoregions. **Results:** In total, 7394 DNA barcode sequences were generated from 170 morphologically identified species (70% of the Nearctic Lycosidae), representing 239 Barcode Index Numbers (BINs). Adult specimens were not available for 19 BINs and were assigned interim names. In total, 30 species shared BINs, two were distinguished with shallow (2%) intraspecific sequence divergence (i.e., BIN splits). Seventy of the 75 Nearctic World Wildlife Fund ecoregions were represented by 1–42 BINs (and 11 represented by >20 BINs), which permitted a thorough exploration of distributional patterns. **Significance:** We present a comprehensive DNA barcode reference library for over two-thirds of the Nearctic wolf spider fauna. The results confirm that DNA barcodes are an effective tool for the identification of Nearctic wolf spiders. With the exception of several cases of potentially cryptic species, BINs and species correspond well, suggesting that rapid and accurate estimates of spider diversity are possible with this approach. It has also permitted the first attempt to resolve the distributional pattern for this large Nearctic family of spiders.

Dating African savannas

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A common assumption is that climate determines the major vegetation zones of the world. Africa is the grassiest continent with extensive savannas dominated by C4 grasses. It is the largest global anomaly to the climate assumption as many savanna regions are wet enough to support forests. The anomaly is most commonly explained as due to human deforestation by burning and felling. This implies the grassy vegetation is of recent anthropogenic origin. This view has led to policies for extensive

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restoration of "degraded, deforested" landscapes motivated, in part, by carbon sequestration that might reduce global warming. Fires are very common in the wetter savannas accounting for ~70% of global annual burnt area. In drier savannas, large mammal herbivory is (was) important in reducing woody plant populations. To explore the key question of the antiquity of tropical grassy vegetation, we used dated phylogenies of an extensive sample of woody plants derived from DNA barcoding. We explored the origin of fire-dependent savannas by focussing on "underground trees", a peculiar growth form unique to frequently burnt savannas. To explore the origin of heavily browsed savannas, we focussed on spiny plants. Spines are a very common structural defence against common forms of mammal browsing. The phylogenetic analyses have revealed that grassy vegetation in Africa is millions of years old, and far older than human deforestation. These studies therefore challenge the prevailing deforestation hypothesis. They are also offering novel insights into how and why savannas evolved from a minor vegetation type to a major global biome covering nearly a quarter of the world's land surface.

DNA barcoding as a vehicle for biodiversity mainstreaming: a path towards sustainable future

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Background: DNA barcoding and iBOL have boosted taxonomy and biodiversity science through helping transfer knowledge and technological advances across national borders. This was reaffirmed by the endorsement of the 13th CBD Conference of the Parties (2016); however, uptake outside academia remains limited. Researchers in many lower-income countries are heavily underfunded; in other nations, they rely on government-funded academic grants, which tend to favour innovation over well-established practices. Despite the growing acknowledgement of the importance of "mainstreaming" biodiversity into sectoral and cross-sectoral development strategies, attaining a growing stakeholder base and sustained support poses a serious challenge. **Results:** National Barcode of Life networks can help mainstream biodiversity by leveraging the potential of DNA barcode applications, but they would need to broaden their scope and mandate. The new strategy has to be more inclusive of practitioners outside academia hampered by the taxonomic impediment. The limited capacity building experience under iBOL has outlined a number of important shortfalls that should be addressed: Targeted awareness raising among prospective stakeholders and development of open information resources; Establishment of globally accessible multi-tier training platforms ranging from distance education for all interested to practical hands-on training opportunities for all qualified; Specialized consulting to facilitate on-site deployment of processing and analytical workflows and, when national funding becomes available, operationalization of national and regional platforms for applied molecular biodiversity surveillance. International development agencies can play a key role in facilitating regional connections between nations with technological or resource disparity to ensure that operations in under-resourced nations could take advantage of the infrastructure and technology available to their neighbours. **Significance:** As DNA barcoding matures, it loses its appeal as "cutting-edge science"; however, good prospects remain to use it as a vehicle for biodiversity mainstreaming by expanding existing BOL network structures beyond academic research partnerships to address fundamental humanitarian challenges.

Macroevolutionary processes shaping monocots diversity: the new era of phylogenetics

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Background: Evolutionary radiations, patterns of morphological evolution, and historical biogeography are crucial for understanding the evolutionary history of organisms. The recent development of statistical packages, triggered by the increasing amount of DNA sequence data accumulated via barcoding projects (among others), allow us today to (*i*) infer diversification dynamics of lineages by locating shifts in diversification rates and identifying radiating clades, (*ii*) reconstruct historical biogeography using increasingly large molecular datasets, (*iii*) model the evolution of intrinsic and extrinsic factors through time, and (*iv*) disentangle the very complex interplays between earth's history and lineages' evolution. **Results:** Here, I will present recent studies conducted on grasses (Poaceae) and restios (Restionaceae) of southern Africa using these newly developed methods. I will show that it is now feasible to gain insights on the mode and tempo of evolution of lineages and biological traits in relation with paleo-ecology and climates. **Significance:** This opens up a new era in macroevolution thanks to (*i*) the increasing amount of molecular data produced, (*ii*) the tendency to comprehensive species-level phylogenies inferences, and (*iii*) the development of complex and powerful statistical models.

Standardized biodiversity assessments using next-generation sequencing

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Background: DNA barcoding is a powerful tool for cataloging biodiversity. While Malaise traps are commonly used to assess insect diversity, traditional approaches used to identify Malaise trap specimens are time consuming and, despite the substantial reduction of Sanger sequencing costs, remains expensive. Next-generation sequencing (NGS) can reduce the cost and time burden to analyze Malaise trap samples, and lead to the rapid assessment of insect biodiversity using a metagenomic approach. We compare the taxonomic resolution of fragments of different length using the Illumina MiSeq and Ion Torrent (PGM and S5) platforms. We also analyse the effect of tissue type, lysis time, and DNA polymerase on species recovery and (or) sequencing error rate. **Results:** This study demonstrates that longer barcoding fragments (i.e., 462 vs. 407 bp) improves taxonomic resolution in metagenomic samples. Illumina MiSeq reads have lower rates of insertion and deletion errors than the more cost efficient Ion torrent, but the error rate of the latter is reduced with the use of a high-fidelity polymerase. Ion torrent and Illumina have similar specimen recovery and coverage and most specimens were recovered with a subset of 150 000 reads. There is no difference in specimen recovery from different tissue types (leg or abdomen), but PCR bias is prevalent in pooled DNA sources with a few specimens dominating read coverage (<1000×). There are few differences in genera recovery between different lysis times of 1 h (91.8%), 2 h (91.3%), 4 h (93.25%), 8 h (89.9%), 16 h (94.2%), and 32 h (93.25%). **Significance:** This study highlights the influence of barcode fragment length on taxonomic resolution for metagenomic studies. The method of sample preparation (bulk vs. individual lysis) influences the effect of primer bias on diverse taxonomic samples. This work develops standardized protocol for inventorying insect biodiversity using Malaise traps.

Expanding on barcode data: genome skimming and organellar genomes

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Background: By revolutionising our capacity to capture sequence data, high-throughput sequencing (HTS) has enabled DNA barcoding to be used in metagenomics applications like ecological surveys, forensic applications, and reference library construction. Because of its cost efficiency, HTS also allows the acquisition of "super barcodes" or whole organellar genome sequences, and enables genome skimming to further explore the impacts of evolutionary variables, such as reproductive sys-

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tems and parasitism, on GC content, codon bias, and rates of molecular evolution. As a test of super barcoding, we targeted traditionally difficult-to-resolve groups (i.e., willows) and those with elevated rates of nucleotide substitution (i.e., scale insects). **Results:** We use second- and third-generation sequencers to assemble whole organellar genomes from fresh specimens and for genome skimming archived DNA extracts. We compare the use of targeted enrichment protocols for organellar DNA versus genome skimming with established DNA libraries. Preliminary results indicate that a targeted enrichment approach increases the number of organellar sequences and allows increased multiplexing, reducing costs. By comparison, a genome-skimming approach captures nuclear regions along with the coding portions of organellar genomes, allowing for a broader examination of genomic features in the target taxa. Both approaches allow us to capture sufficient sequence data to provide a critical test of "super barcoding". **Significance:** As new HTS platforms emerge, DNA barcoding will expand in scope and utility. This study evaluates the possibility of augmenting DNA barcoding standards to include additional genomic regions from HTS data. The use of HTS allows the exploration of evolutionary trends, revealed by barcoding analysis, in non-model organisms.

Linking aerial grass pollen biodiversity and human health: an environmental genomic approach

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In Europe and the UK, grass pollen is the single most important outdoor aeroallergen; 27% of the population are sensitised to grass pollen. Grass pollen allergy has been linked to increased risk of allergic asthma exacerbations, which can lead to hospitalisation and fatalities. Sensitivity towards grass pollen varies with species, of which there are over 150 in the UK. However, due to few unique morphological features, grass pollen from different species cannot be discriminated using traditional observational methods. Currently, there is no way of detecting, modelling or forecasting the aerial-dispersion of pollen from the biodiversity of UK grasses and so they are coalesced into a single group in the UK forecast. PollerGEN is an interdisciplinary NERC project with the aim of revolutionising the way that pollen dispersion is measured and forecast, and to increase our understanding of the ecology of aerial dispersed pollen. In collaboration with the UK Met Office, a key goal of the project is to build a more accurate forecast of individual grass pollen species. Using environmental genomics (shotgun sequencing, DNA metabarcoding, and qPCR), we will identify which species of grass pollen are present during the summer months across 16 specific collection sites in the UK, with the aim of measuring the abundance of different allergenic species of grass. The information will be used to model the spatial and temporal deposition of grass pollen and identify linkages to human health. The project therefore aims to provide a paradigm shift in our understanding of the ecology of windborne pollen in time and space and inform the public about the timing and environmental factors that put them at risk of exposure to pollen they are allergic to, a key strategy in the prevention of allergy and asthma attacks.

Check out bioliteracy: novel partnership with a city library system to expand DNA barcoding initiatives in a global biodiversity hotspot

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Background: Since 2013, the San Diego Barcode of Life (SDBOL) created unique academic, private, corporate, and public partnerships, contributing >50 000 DNA barcode sequences from San Diego County to the Barcode of Life Data System (BOLD). Public libraries are devoted to civic literacy, with proven infrastructure for public engagement, education, and continuity across diverse citizenry. The City of San Diego Library is nationally recognized for innovative science programs. San Diego, the eighth largest city in the USA, is a biotechnology hub, and its leadership is committed to building its "Smart City" stature. Citizen Science networks are key participants in existing science education outreach. We conceived a novel library bioliteracy campaign to scale distribution of LifeScanner (LS) barcoding kits and Global/School Malaise Program (GMP/SMP) traps from the Center for Biodiversity Genomics (CBG), stimulating interest to leverage existing relationships and BOLD data to advance mutual goals. **Results:** In 2015, SDBOL and the Library piloted distribution of 20 LifeScanner kits and three SMP traps at library sites. In March 2017, leading the City's "STEAM in to Spring" initiative, the Library's "Catalog of Life" project distributed 1000 LS kits to 36 geographically distinct library sites. We updated LS mobile device app and websites to facilitate participation. Existing protocols and web portals supported GMP/SMP placement at 10 libraries, with overall goal to collect over 4000 specimens for barcoding. City STEAM infrastructure contributed materials, personnel, outreach, and leadership to roll out and sustain the program with dozens of insect- and biology-themed speakers and events. Library checkout management and LS tracking were key tools to scale the design, execution, and assessment of the project. **Significance:** We believe ours is (*i*) the first initiative to leverage public library infrastructure to engage citizens to expand and use a global DNA barcode reference library, and (*ii*) the largest single distribution of LS technology.

Using sedaDNA alongside palaeoenvironmental proxies for understanding wetland and lakeside archaeological sites

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Wetland sites, including settlements on lake shores and artificial islands, often provide a wealth of well-preserved archaeological material, but they are generally difficult and expensive to excavate conventionally. An alternative, or complimentary approach, can be the retrieval of archaeological data from lake sediments, which can under certain conditions contain a continuous record of the archaeological site, the lake, and its surrounding catchment. Here, we present early data from a study of three crannogs (artificial island settlement) and an Iron Age lakeshore village in Scotland where sedimentary ancient DNA (sedaDNA) data was analysed from proximal sediment cores. The sedaDNA provides detailed information about the plants and mammals that lived, died, or were kept on the sites in different periods of site use. This information is compared with a range of traditional palaeolimnological proxies that allow us to differentiate between (*i*) changes that happened regionally in the lake catchment (based on pollen, x-ray fluorescence scanning, stable carbon and nitrogen isotopes, n-alkanes); (*ii*) changes that happened in the lake ecosystem (based on loss-on-ignition, diatoms, biogenic silica, invertebrates, C:N ratios); and (*iii*) changes that occurred very locally at the sites (based on pollen and spores, invertebrates, sterols, PAHs, and sedaDNA). Our sedaDNA results complement data from both archaeological excava-

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tion and traditional palaeo-environmental proxies to provide a more detailed and robust image of the environment in which our ancestors were operating. We also show that different proxies in the same sediment core provide insights in past environments at different spatial scales.

Flying insects in the southern Atlantic Forest: striking biodiversity and diverse temporal demographic patterns

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Background: The Atlantic Forest in South America is one of the most biodiverse ecosystems on Earth, harboring around 7% of the species of our planet. Because this biodiversity hotspot possesses high rates of endemism and has been extremely disturbed, it is a priority for conservation efforts. Arthropods are good indicators of biodiversity and ecosystem integrity of forests. Therefore, a comprehensive assessment of their species composition through time and space is crucial for biomonitoring. DNA barcoding can support this activity. In the context of the Global Malaise Program, Argentina deployed a Malaise trap in Misiones province, in the southern Atlantic Forest, and insects were collected weekly for a year starting in February 2013 to evaluate local diversity and patterns of temporal succession. **Results:** Overall, 75 589 specimens were collected (67 565 barcode sequences recovered). These represented 8753 Barcode Index Numbers (BINs) (proxy for species), of which 81% had not been previously barcoded. Diptera was the most abundant order (76%), followed by Hemiptera (7%) and Lepidoptera (5%). We assessed the temporal distribution of the 38 most abundant BINs, which constitute conspicuous components of this ecosystem and identified six clusters of BINs with similar time series distributions. Each of these clusters included representatives from different orders, showing that demographic patterns through time are not order specific. We also analyzed which environmental variables (temperature, precipitation, wind speed) can best explain the abundance distribution of each BIN. **Significance:** Thousands of new species were added to the DNA barcode library. In addition, we dramatically increased access to biodiversity information for this region (currently the Global Biodiversity Information Facility (GBIF) only includes 321 species of insects in the area). Based on these results, the possible role of these BINs as biological indicators for this ecosystem can be further investigated, supporting both biomonitoring and the assessment of changes in community structure due to ongoing climate change and environmental disturbance.

Comparison of whole genome to 16S sequencing analysis of intestinal microbiome in Argentinian children with helminth and protozoa infections

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Next-generation sequencing (NGS) for microbiome analysis is commonly performed using 16S rRNA gene sequencing or whole-genome shotgun (WGS) sequencing. We carried out both WGS and 16S sequencing on human fecal samples from a 122 Argentinian cohort study focusing on two groups: helminth infected (*Ascaris*, *Ancylostoma*, *Necator*, *Strongyloides*, and *Trichuris*) versus non-infected (no-parasite) individuals verified by multi-parallel real-time quantitative PCR. WGS approach provided higher resolution allowing classification to the bacterial strain level and in some cases even sub-strain level. 16S sequencing could not provide resolution below genus level. Both methods demonstrated similar sensitivity to detect Shannon alpha diversity differences. While there were no statistical differences within the helminth infected group (p=0.999) or no-parasite group (p=0.400), WGS showed a significant increase in difference of means (DOM) as compared to 16S rRNA gene sequencing. DOM provides a measure of the change in proportion of specific bacterial sequences for helminth and no-parasite groups. This measure is useful for determining the capacity of an assay to discriminate between two experimental groups and small effect size. The WGS method provides rich metagenomic functional information as compared to 16S rRNA sequencing. Metagenomic functional information for 16S rRNA reads can be inferred using PICRUST software through taxonomic information, but it lacks the direct evidence of genes found in WGS. On the other hand, 16S sequencing is computationally inexpensive, while WGS data are challenging to manage/analyse and require software with complex algorithms. Our results show that WGS offers higher taxonomic resolution and discrimination along with metagenomic functional information, while 16S provides a reasonable option if the taxonomic information is the primary focus of a study. This study provides important information for selecting the optimal assay based on function and price with implications in evolutionary investigations and tropical medicine.

Assessing DNA barcode as a diagnostic tool for timber species of *Dalbergia* (Leguminosae) in Mexico

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Background: The genus *Dalbergia* is a pantropical group with around 250 species. In México, *Dalbergia* comprises 20 species, six of which are endemic. *Dalbergia*, or rosewood, are distinguished by their heartwood which is of high economic value, due to its beauty, durability, and excellent physical, mechanical, and acoustic properties. They also produce metabolites, used as antimicrobial, antifungal, antibiotic, antioxidant, and cytotoxic agents. Several species of the genus are used in the timber industry and thus subject to severe exploitation and illegal international trade. The species populations are at great risk due to extensive habitat loss and fragmentation. Barcoding the species could aid in the monitoring of *Dalbergia* and its trade. **Results:** In this project, we investigated the potential of herbarium specimens to generate DNA barcodes of the Mexican species of *Dalbergia* and compared their identities against timber samples. Small fragments of leaf material were taken from 48 herbarium specimens housed at Mexico's National Herbarium. A total of 29 species were processed using standard protocols for DNA extraction, amplification, and sequencing. Timber samples were processed using the MagMax Plant DNA (Invitrogen) extraction kits with PVP 40 2% (*v*/*v*). The amplification and sequencing were carried out using standard protocols. Core barcodes *rbcL* and *matK* were used, with the additional ITS nuclear region. The success rate was higher for *rbcL* (93.75%) than for *matK* (91.67%) and ITS showed 100% success rate. Complete standard barcodes were obtained for 91.67% of the 48 samples; 86.21% of the species. Two regions, *rbcL* and *matK*, were sequenced for two timber samples. The consensus sequences were searched on BLAST and the result proved positive for *Dalbergia*. **Significance:** This is the first DNA barcode reference library for the Mexican species of *Dalbergia* and highlights DNA barcoding as a tool to detect and combat the illegal trade of species of *Dalbergia*.

Detection of invasive freshwater fish in lakes in British Columbia, Canada, using eDNA metabarcoding

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Background: Freshwater ecosystems globally are under intense pressure from multiple stressors including invasive finfish. Invasive fish species pose major threats to freshwater biodiversity and can nega-

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tively impact economically-important activities including commercial fisheries, sport fishing, and tourism. Invasive fish can increase predation pressure on native species, cause habitat destruction, and alter the structure of natural food webs. Early detection maximizes the chance for effective management intervention towards preventing or mitigating potentially severe negative impacts. Environmental DNA (eDNA) metabarcoding offers a hitherto unprecedented opportunity for time and cost efficient high-sensitivity molecular monitoring of several known invasive species simultaneously, while also enabling the detection of new incursions. Further, it can serve as a biodiversity monitoring tool to assess changes to fish community assemblages in freshwater environments in response to other stressors. **Results:** We tested the effectiveness of various eDNA sampling and filtration procedures for invasive finfish detection by metabarcoding at one lake in British Columbia, Canada, with well-known fish assemblage data acquired by traditional means. We also assessed variability in detection success between summer and autumn. Three eDNA metabarcoding markers, all in mitochondrial 12S, were tested and results showed that one marker alone can provide confident detections and species-level discrimination. To facilitate accurate taxonomic assignments and maximize invasive species detection probabilities, reference sequences for 12S were generated from an existing DNA barcoded specimen collection representing 96% of Canadian freshwater fish (194 species; >800 samples) and from known invaders. **Significance:** The optimization of field protocols and the field validation of eDNA metabarcoding for invasive freshwater fish detection in British Columbia makes this tool readily available for ongoing monitoring and management of high-risk invasive fish present in this region. The secondary spread of these species pose direct and serious threats to the persistence of economically- and culturally-important native Pacific salmon stocks.

DNA barcodes help to identify sandflies in conserved areas from Norte de Santander (Colombia)

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Background: Prediction of possible epidemic outbreaks or emergence of infectious diseases from wild areas is a relevant task for disease ecology. In particular, arthropod-borne transmission is a fundamental factor in increasing the incidence of emerging infectious diseases. Forest fragmentation and landscape changes by anthropogenic disturbances can change the transmission dynamics of viruses, bacteria, and protozoa due to exploitation of humans and domesticated animals as novel hosts. In this study, we made an inventory of sandflies of the genus *Lutzomyia* in a Sub-Andean forest in the Pamplonita River basin. We used DNA barcodes for molecular identification of the *L. verrucarum* group. **Results:** Specimens collected with CDC-light and Shannon traps were identified using morphological keys. Pieces of the thorax or abdomen were used for DNA extraction and PCR following the protocols proposed by the DNA barcode initiative. Sixty-two (62) sequences belonging to sandfly species of the *L. verrucarum* group (*L. spinicrassa*, *L. shannoni*, *L. pia*, *L. youngi*, *L. ovallesi*, and *L. longiflocosa*) were obtained and used for estimating nucleotide diversity, K2P genetic distances within and between species, defining haplotypes, and inferring a neighbour-joining dendrogram. The interspecific genetic distances ranged from 0.065 to 0.219. A barcoding gap was found between the analyzed species. We found polymorphic sites and high haplotype diversity. The NJ dendrogram showed six MOTUs corresponding to the species identified morphologically and confirmed the presence of these species in the area sampled. DNA barcodes separated the species and confirmed morphological identifications, and enabled the identification of incomplete or damaged specimens. **Significance:** DNA barcoding is an alternative tool for confirming the taxonomic assignments in species with similar morphological features, such as the *L. verrucarum* group. However, it is important to increase the species sampling and study specimens of the same species from different geographical locations in order to explore the utility in phylogenetic and phylogeographic studies.

Investigating bird strikes in Brazil through DNA barcoding

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Background: Bird–aircraft collisions, or bird strikes, are frequent worldwide, causing huge material losses and endangering the lives of crews and passengers. Identification of birds involved in such events can be important for many reasons, including the assistance of accident investigations and supporting management plans to reduce risks in critical areas. Bird strike remains are frequently fragmented or restricted to blood stains, which makes morphological identification impossible. In these situations, DNA barcoding can be used to associate unknown samples to reference samples by comparing cytochrome *c* oxidase I gene (COI) sequences. The Barcode of Life Data System (BOLD) provides a reference database with authenticated sequences and a searching tool suitable for species identification. **Results:** Between 2014 and 2016 the Brazilian Federal Police DNA Laboratory received for analysis 53 unidentified bird strike samples sent by CENIPA, the Brazilian Air Force unit responsible for the investigation of aeronautical accidents in Brazil. Using BOLD and species occurrence data it was possible to identify 49 samples (92.5%), all but three to species level. Unsuccessful identifications were due to the lack of DNA in the samples or degradation. Thirteen bird species or genera were identified: the black vulture (30.2%), the southern caracara (22.6%), the magnificent frigatebird (9.4%), and the southern lapwing (7.5%) represented most of identifications. Two different species were recovered from one of the samples, possibly due to two independent impacts in the same part of the aircraft. **Significance:** Although a more extensive study is necessary to corroborate the results presented here, apparently a few species are more frequently involved in bird strikes. In order to be more cost effective, management plans should prioritize these species. As showed, the use of DNA barcoding associated to species occurrence is an efficient and sometimes the only way to identify bird strike remains, helping to improve flight safety in Brazil.

Effect of access to human-subsidized food on bacterial gut microbiome of the Neotropic cormorant (*Phalacrocorax brasilianus*)

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Background: Aquatic birds represent one of the most abundant and ubiquitous avian species and are natural reservoirs for a large variety of emerging pathogens. The Neotropic cormorant (*Phalacrocorax brasilianus*) is one of the most abundant marine birds of the Americas, using different natural as well as urban habitats. In the present study, we employed DNA metabarcoding to explore the bacterial gut microbiome from two cormorant populations in Chile: Valdivia population, which uses natural and urban spaces and is subsidized by the local fish market; and Chillan population, which feeds exclusively on fishes from natural habitats. **Results:** After MiSeq sequencing and QIIME analysis, we found 2633 bacterial operational taxonomic units (OTUs) using the 16S rRNA: 79% of the OTUs were shared between both populations, whereas 14% were exclusive for Chillan and 7% were exclusive for Valdivia. We also found that 615 from 2633 OTUs (23.4%) showed significant differences in their relative abundances between populations. Interestingly, *Campylobacter* exhibited a higher relative abundance in Valdivia (75.6%), whilst *Pepstreptococcus*, *Coprococcus*, and *Clostridium* were more abundant in Chillan. Richness and phylogenetic diversity did not differ between populations, but community structure did. PICRUSt analysis revealed that functional composition differed between populations, and genes related to immune diseases and

biosynthesis of secondary metabolism were more abundant in Chillan population, whereas genes related to catabolism and signal transduction were more abundant in Valdivia population. **Significance:** DNA metabarcoding provides a straightforward approach to study microbiomes associated to wildlife. The Neotropic cormorant seems to be a natural reservoir of *Campylobacter*, a foodborne bacterial pathogen, which has an astonishing abundance in the aquaculture-subsidized Valdivia population. Antibiotic use by the aquaculture industry can speed up the development of antibiotic resistance in *Campylobacter*. Consequently, antibiotic resistance could have significant effects on the community structure of gut microbiome in wild birds and on the dispersal of antibiotic-resistant bacteria.

Phyllosphere microbiome associated to vineyards and native forest in the Mediterranean ecosystem from Chile

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Background: Mediterranean ecosystems are biodiversity hotspots, where vineyards are common components of the landscape. However, increasing land and water demands by vineyards have augmented the threats on these ecosystems. Conservation of these biomes is not only important because their biodiversity values, but also because they can provide ecosystem services to human activities such as wine production. In the present study, we employed DNA metabarcoding to explore bacterial and fungal communities inhabiting the phyllosphere of *Vitis vinifera* under conventional and organic management, and also those communities inhabiting the phyllosphere of adjacent native forests in central Chile. **Results:** After MiSeq sequencing and QIIME analysis, we found 4882 bacterial and 897 fungal operational taxonomic units (OTUs), using the 16S rRNA and ITS2 as genetic markers, respectively. Interestingly, 83% of the bacterial OTUs and 96% of the fungal OTUs were shared between forest and vineyard samples, suggesting that some ecological functions could be present in both habitats. Additionally, OTU richness and diversity were different among habitats: bacterial indices were higher in vine leaves, whereas fungal indices were lower in vine grapes. Bacterial and fungal community structures were different among habitats: forest samples form a separated cluster, whereas vineyard-related samples overlap in the NMDS space. On the other hand, bacterial and fungal community structures changed between agricultural managements, with this effect more evident in vine leaves. **Significance:** DNA metabarcoding provides a straightforward approach to study microbiomes in threatened ecosystems such as Mediterranean biomes. At the same time, this approach can work as a biomonitoring tool for detecting beneficial (lactic bacteria and fermenting yeasts) and detrimental (acetic bacteria and pathogenic yeasts) microorganisms for winemaking. We consider the implementation of environmentally friendly practices by the wine industry helpful for sustainable wine production and for maintaining the microbial diversity and ecosystem functions associated with natural habitats.

DNA barcoding for the identification of endangered plants with commercial use: the case of *Gentianella* spp. *"hercampuri"* (Gentianaceae) from Peru

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Background: Peru is a country that is rich in both biological and cultural diversity, where the use of plants in popular medicine is deeply rooted and not only exists in rural areas, but has reached major cities and international markets alike. Many species of wild flora that are harvested for medicinal purposes come from the Andes, a region that harbors a great number of endemics. One of the main concerns is the little importance given to the correct identification of these plant species by traders and regulators. *Gentianella* (Gentianaceae) is a genus with a high percentage of endemism at a national level (74%). It includes four species (*G. alborosea*, *G. nitida*, *G. thyrsoidea*, and *G. tristicha*, mainly from Ancash, Junin, and Huanuco), which are exploited for their medicinal properties, and they are frequently commercialized under the single species name *Gentianella alborosea* ("*hercampuri*"). As part of a larger study on phylogenetic relationships within the genus, as well as to develop practical tools for the identification of commercialized species, ITS, *rbcL*, and *matK* barcode markers were sequenced and analyzed. **Results:** Preliminary results indicate that barcoding markers alone are not able to resolve relationships among some of the species within *Gentianella*, and that additional markers (e.g., entire *trnK/matK*) are required. Furthermore, difficulties in defining species boundaries due to morphological gradients, which attribute to hybridization, further confound the relationships. **Significance:** The results will provide valuable information for the accurate identification and responsible management of endangered species traded on the commercial market.

DNA barcoding for the identification of a new species of sea cucumber from the Colombian Caribbean

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Background: Sea cucumbers from the order Aspirochirotida are among the most diverse of the holothurians. Representatives of the Stichopodidae are widely distributed in the Caribbean sea. *Isostichopus* sea cucumbers are widespread in the entire Caribbean region, but little is known about the native species. For the Colombian Caribbean only one species, *Isostichopus badionotus*, has been reported. The present study describes the use of DNA barcoding as a complementary tool to identify a new species of *Isostichopus* from the Colombian Caribbean Sea. For this purpose 27 individuals of three different morphotypes were dissected and preserved in absolute ethanol (99.5%). A 665-bp fragment of the COI gene and 508-bp fragment of 16S were amplified and sequenced, and, along with other sequences downloaded from GenBank, sequence divergences were calculated using the K2P distance model. A neighbour-joining (NJ) tree and a distance matrix were generated using MEGA 6, and Bayesian analyses were performed using MrBayes. **Results:** Two genetic lineages of *Isosticopus* were represented in our samples. One lineage is *I. badionotus*, and another is *Isostichopus* sp. *aff badionotus*, not yet described. Both the NJ and the Bayesian analyses recovered the same tree topology. The distance matrix indicated that the average intraspecific distance was 0.20%, while the average interspecific distance value was of 7.80%. Comparisons with *S. herrmanni* sequences indicated that average intergeneric distance value was of 16%. **Significance:** It is well known that studies that confirm the taxonomic classification of species are an essential tool for the implementation of management plans and conservation programs for sea cucumbers, and that morphological descriptions should be complemented with barcoding analyses. In this study, the barcoding results, corroborated by the morphological analyses, indicated that there are two different species of *Isostichopus* in the Colombian Caribbean.

What's in a name? Unravelling the species diversity underpinning the global "snapper" trade

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Background: The snappers, family Lutjanidae, are one of the most highly prized and important circumtropical marine fisheries re-

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sources, with various species traded fresh and frozen across the globe. However, given their high commercial value, many other diverse species may masquerade under the umbrella term snapper on global consumer markets. This study sought to harness the power of DNA barcoding to reconstruct the first "global map" of the biodiversity underpinning the international snapper trade, elucidating rates, patterns and complexities of labelling accuracy, as well as linking the outcomes with trade data and regional regulations, to identify variables responsible for these patterns. **Results:** Following collection of samples from geographically widespread markets within Europe, North America, Oceania, Asia, and Africa, DNA barcoding revealed that only ~65% of "snappers" could be assigned to the family Lutjanidae. The remainder were identified as members of at least 10 other fish families, including seabreams (Sparidae), threadfins (Nemipteridae), alfonsinos (Berycidae), fusiliers (Caesionidae), sea basses (Serranidae), rockfishes (Sebastidae), emperors (Lethrinidae), scorpionfishes (Scorpaenidae), armorheads (Pentacerotidae), and grunts (Haemulidae). "Snapper" samples from Australia and New Zealand comprised largely of sparids, whereas in the United Kingdom—which follows the world's most stringent seafood labelling regulations—~50% of collected "snappers" were deemed mislabeled. **Significance:** The results of this study demonstrate that generic market labels such as "snapper" represent one of the most at-risk denominations in the context of global seafood traceability, hampering consumer choice and market control by grouping species together for sale with different values, conservation concerns, and even health impacts. Bringing this trade into the open should strengthen the case for a revision of international regulations and guidelines that permit the use of such umbrella terms, laying the groundwork for more accurate labelling and essentially more transparent and sustainable seafood markets.

DNA barcoding using cytochrome *c* oxidase I (COI) and recombination activating gene 1 (RAG1) cannot discriminate between *Sardinella tawilis* and *S. hualiensis* (Clupeiformes: Clupeidae)

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Background: This study analyzed species boundaries within the genus *Sardinella*, focusing on the relationship between *S. tawilis* and *S. hualiensis*, the latter only recently reported from Philippine waters. Since fishes of the genus *Sardinella* are all marine, except for the *S. tawilis*, previous studies were designed to determine the closest marine relative of this Philippine-endemic species. *Sardinella hualiensis* was found to be morphologically similar to *S. tawilis*, so further molecular analysis was required to determine their relationship. **Results:** Mitochondrial cytochrome *c* oxidase I (*COI*) gene was used in this study. A neighbour-joining tree was constructed using sequence divergence values determined by the Kimura 2-parameter model. A single clade with 100% bootstrap support was formed, and the interspecific divergence ranged from 0% to 0.5220%, which is clearly below the suggested 3%–3.5% cutoff for species discrimination. An interesting outcome is the 0% genetic distance between *S. tawilis* and a Taiwanese specimen of *S. hualiensis*. Incipient allopatric speciation is a possible explanation for the low genetic distance between *S. hualiensis* and *S. tawilis*. A nuclear gene region, recombination activating gene 1 (*RAG1*), was used to further validate the findings of the study. Low interspecific genetic distances (0% to 1.1714%) provide additional evidence that *S. tawilis* and *S. hualiensis* belong to a single species. **Significance:** A more thorough understanding of the genetic status of *S. tawilis* could help with sustainability and management studies for this fish species, as it is the main source of income and livelihood for most of the locals in the Taal Lake area, where *S. tawilis* is endemic.

Metagenomic analysis of cattle egret (*Bubulcus ibis* L.) specific gut microbiota

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Background: The cattle egret (*Bubulcus ibis* L.) belongs to the family Ardeidae, and is found ubiquitously in the tropics, sub-tropics, and warm temperate regions. Its ecological plasticity for foraging is one of the main reasons for its success in a wide range of environments. Cattle egret harbors a diverse range of microorganisms in its alimentary canal, which play an important role in the well-being of the bird. The gut microbiota process and extract nutrients present in their host's diet, develop the immune system, recycle organic compounds, minerals, and water. In our study, next-generation sequencing (NGS) technology has been carried out on the fecal pellet to analyze the microbial community within the cattle egret by isolation and polymerized chain reaction using 16S rDNA primers (16S rDNA F-GAGTTTGATCCTGGCTCAG and 16S rDNA R-ACGGCTACCTTGTTACGACTT). **Results:** The data generated by the study contains 56 318 sequences totaling 76 431 398 base pairs with an average length of 136 bps. A total of 12 bacterial phyla, 21 classes, 37 orders, 76 families, 119 genera, and 234 species were examined by comparing sequences against the Ribosomal Database Project (RDP) via metagenomic RAST (MG RAST) server. Firmicutes were found to be the most abundant and diverse phylum. They include a wide range of uncultured organisms, and some members of this phylum are responsible for the degradation of starch and cellulose. We also observed the presence of Ptoteobacteria, Actinobacteria, and Firmicutes within the cattle egret. **Significance:** The present study envisages examining the role of symbiotic microorganisms in nutrition, development, and regulation of a candidate bird species. NGS has proven to be a powerful tool for understanding the diversity and functional capacity of a range of microbes within an organism.

DNA barcoding of seafood reveals a low rate of mislabeling in Qatar

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Background: DNA barcoding techniques have made it possible to authenticate various species used for food and medicinal purposes. In the identification of seafood species, studies are concentrated in North America and Europe. Elsewhere, including countries in the Middle East and North Africa, studies of this sort are scarce. For a growing country such as Qatar that relies on imports for the majority of its food supplies, the increasing demand calls for authentication of seafood in particular as an alternative to red meats favored by its population known to show high rates of cardiovascular diseases. **Results:** This student-centered research focuses on fish fillet available at 10 major supermarkets in Doha, Qatar. A cocktail of eight primers attached with M13 tails established for fish species identification was adopted to facilitate PCR and sequencing. Sequences were compared with those available in GenBank and the Barcode of Life Data System (BOLD). Among the 38 unique fish fillet packages available in the markets, only two are determined to be mislabeled, a rate of about 5%. **Significance:** This study is the first of its kind conducted in countries in the Arabian/Persian Gulf region and one of the only four known countries in the Middle East and Northern Africa in which seafood authenticity has been investigated. The relatively low rate of mislabeling in the samples perhaps is due to strict local food safety regulations, which may have led to high consistency between the package labels and their contents.

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Complete chloroplast genomes of medicinal plants: *Dioscorea opposita* and *D. collettii*

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Background: *Dioscorea* L. has a long history in China as medicinal and edible plants, which have important economic values. However, the identification problem of *Dioscorea* is still unsolved because of the limitations of traditional methods. Flora of China records 52 plants, and some varieties of species are too complex to distinguish from morphology. Previous research showed that the universal DNA barcode sequences cannot identify *Dioscorea* effectively. Solving the identification problem of *Dioscorea* is the vital research content in the post-barcode era. In this study, the complete chloroplast (CP) genomes of *D. opposita* and *D. collettii* were sequenced using Illumina Hiseq X. **Results:** The complete CP genome size of *D. opposita* is 152 960 bp and that of *D. collettii* is 153 869 bp. A pair of inverted repeats (IRs) of 50 986 bp is separated by a large single-copy region (LSC, 83 152 bp) and a small single-copy region (SSC, 18 822 bp) in *D. opposita*. Moreover, a pair of IRs with a length of 51 182 bp is separated by LSC (83 824 bp) and SSC (18 863 bp) in *D. collettii*. Both species contain eight rRNAs and 30 tRNAs, whereas *D. opposita* has 89 protein-coding genes and that of *D. collettii* has 88. The specific DNA regions with high variation will be screened based on the complete CP genomes of *D. opposita* and *D. collettii* that are combined with four other complete CP genomes of *Dioscorea*: *D. elephantipes* (EF380353), *D. rotundata* (KJ490011), *D. nipponica* (KP404629), and *D. zingiberensis* (KP899622). **Significance:** The CP genomes of *D. opposita* and *D. collettii* provide the fundamental basis for the classification, identification, and phylogenetic relationship of *Dioscorea*, especially in screening the specific DNA regions with high variation to identify species of *Dioscorea*. Meanwhile, this research provides enlightenment for solving the identification problem of universal DNA barcodes.

A de novo pipeline for sequencing and assembling amphibians' mitochondrial genomes using NGS technology

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Capture hybridization, long-range polymerase chain reaction (LR-PCR), and high-throughput sequencing are powerful approaches for recovering and assembling the mitochondrial genome (mitogenome), which is an important genetic marker for evolutionary studies. However, gene rearrangements, pseudogenes, and tandem repeats can negatively impact the application of this marker. We developed a new set of primers, a new capture hybridization workflow, and a bioinformatics analysis pipeline for sequencing and assembling 112 mitogenomes from amphibians. In total, the pipeline successfully recovers 83 complete and 14 nearly complete mitogenomes from 53 of 54 genera. The universal primers combined with LR-HY is an efficient approach for recovering complete mitogenomes from the class Amphibia.

The pathology nursery of *Tetracarpidium conophorum* (Mull. Arg.) Hutch & Dalz (Euphorbiaceae) for a sustainable management in the western region of Cameroon

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Tetracarpidium conophorum is a vine of the family Euphorbiaceae producing edible fruits with a wide distribution across West and Central Africa. This liana is classified among multipurpose agroforestry trees providing non-timber forest products with many beneficial properties. Members of the local population transform their nuts into powder to obtain a proteinic food supply and hypocholesterolemian/hypotriglyceridemian vegetable oil. It is also commonly used in traditional medicine to cure several diseases. However, this plant is attacked by some diseases, which destroy root systems and stems. These diseases compromise its regeneration and its facility to improve local populations through its various derived services. The objective of the study was to assess the pathology nursery of this vine to fight against diseases affecting diaspores of nuts. Specifically, we (*i*) carried out a study of plant diversity with the aim of characterizing ecological niche within the study area, (*ii*) evaluated carbon stocks sequestered in cocoa agroforestry systems with the method described by Harriah et al. (2001) to estimate carbon above ground biomass and determine impacts of the diseases on the carbon stock of the vine, (*iii*) proposed strategies to improve management against infectious diseases, pests, or pathogens for the stabilization at the levels of growth and production for local farmers. Results showed that there exist a high diversity of plants which are introduced in cocoa agroforestry systems to better sustain livelihood of local communities and diversify the system. With regards to carbon sequestration, the method of Chave et al. (2005) was used and we obtained aerial carbon biomass data of 10.48, 18.52, and 2.53 tC/ha in cocoa-agroforestry based systems. Urgent actions need to be implemented to identify each pathogenic agent and control diseases in agroforestry species.

Can the marketing of agroforestry tree products and carbon sequestration contribute to livelihood improvement of local populations in Cameroon?

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Tetracarpidium conophorum is a vine of the family Euphorbiaceae producing edible fruits and presenting a wide distribution across West and Central Africa. This liana is classified among multipurpose agroforestry trees providing non timber forest products with a lot of properties. Local population transforms their nuts into powder to obtain a proteinic food supply and hypocholesterolemian/hypotriglyceridemian vegetable oil. It is also commonly used in traditional medicine to cure several diseases. The vine is highly marketed in Cameroon and neighbouring countries while the whole plant is used in cocoa-agroforestry based systems to improve livelihoods and contribution to carbon sequestration. Despite all the benefits of this species, the lack of knowledge on the socioeconomic and ecological potentialities of the vine is a hindrance for sustainable management and improvement of household income for local populations involved in the value chain. The objective of the current study is to assess the socio-economic and ecological potentialities, as well as carbon sequestration potential, in selected agroforestry systems in the Mbam and Inoubou division in Cameroon. Preliminary results demonstrate the high economic potentialities of the resource. Seeds are sold wholesale in 15 kg buckets and cost from US$15.43 to US$27.43 depending on the season of production and amount generated per season. Positive impact of this income had been observed on the livelihood of local population of the area. Regarding the carbon sequestration, the method of Chave et al. (2005) was used to calculate the carbon. An aerial carbon biomass varying from 10.48, 18.52, and 2.53 tC/ha in cocoa-agroforestry based systems was measured. Surveys conducted revealed that wine associated with species in agroforests actively contribute to the sequestration of carbon, thus mitigating the climate change effects. Considering these findings, urgent ac-

tion should be taken for the management of this important vine and its integration in different cropping systems.

ACE-AFBA: DNA barcoding of invertebrates from Antarctic and Subantarctic islands

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The Antarctic Circumpolar Expedition (ACE) occurred November 2016 – March 2017 and was funded by the Swiss Polar Institute. Three legs (Cape Town – Hobart – Punta Arenas – Cape Town) were undertaken on board the Russian Icebreaker *Academik Tryoshnikov* covering the entire circumference of the Antarctic continent. As part of this expedition, the project *A Functional Biogeography of the Antarctic* (AFBA) visited 10 Subantarctic and Antarctic islands in order to assess the diversity of plant and animal life on the islands. Led by Monash University in Australia, this multinational project included researchers and institutes from Australia, New Zealand, South Africa, and the UK. Over 200 soil samples were collected from multiple sites on each of the 10 islands. Here, we present the preliminary results of our efforts to obtain invertebrate DNA barcodes with a particular focus on the soil fauna including Coleoptera (beetles), Collembola (springtails), and Acari (mites). These data will ultimately be used to help understand the history of life in the region, how it will respond to environmental change, and what can be done to secure its future.

DNA and ecological networks: the community barcode approach

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Over the last decade, DNA-based methods for inferring diet and network analyses of ecological systems have exploded with new technologies and analytical techniques emerging faster than manuscripts can be published, with a strong emphasis on the application of DNA barcoding as the main technique. This has generated excitement among a variety of research communities and where species niche requirements are fundamental to understanding community response. However, these developments also present significant challenges in field, laboratory, and analytical systems. It is often a race to keep up with developments rather than a solidification and validation of techniques. How then are we to actually apply these methods to real systems and address real questions? My research group will provide examples of analyses conducted across a diversity of habitats including deserts, tropical jungles, temperate forests, and agricultural landscapes that cover antagonistic, mutualistic, and parasitic interactions addressing species co-existence, the conservation impacts of climate changes like El Niño, and species response to forest modification. We will particularly focus on terrestrial systems involving bats, bees, plants, and insects. In all cases we shift from "diet analysis" to "network analysis". This represents not just a scaling up of techniques, but presents very novel challenges we must consider if we are to truly measure and quantify communities of interactions between predators and prey, the dispersal of seeds and pollen, and the relationship between parasites and their hosts.

DNA barcoding of Great Salt Lake invertebrates

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Background: The Great Basin is the largest endorheic watershed in North America, encompassing an area of 295 000 km². Among the dominant features of this extensive region is Great Salt Lake (GSL), in northern Utah. GSL is the fourth largest terminal lake in the world, with a surface area of 4200 km² and an average elevation of 1280 m. The lake is a fascinating example of an extreme environment, with salinities in some regions exceeding 20%. Invertebrates, including brine shrimp and brine flies, are the only animals that live and reproduce in the lake's waters. However, there are no published studies of invertebrate diversity associated with habitats adjacent to the lake shoreline. **Results:** This study examines the diversity of GSL invertebrates using cytochrome *c* oxidase I (COI) DNA barcodes. Barcodes were generated from 100 samples collected from various regions of the lake. While known brine fly species were occasionally identified, the survey revealed a large number of taxa not previously described from the GSL ecosystem. Insects are the dominant group, but barcodes were also obtained from arachnids, nematodes, and crustaceans. Values for intraspecific sequence variation among insects were examined and compared to variation between congeneric species. **Significance:** The GSL ecosystem is one of the western hemisphere's principle bird habitats, and the lake supports a population estimated at 7.5 million individuals distributed among 257 species. While brine shrimp and brine flies are thought to be the principal food source for the birds, it is likely that they are utilizing other invertebrates as well. This is the first systematic study of invertebrate diversity associated with the GSL ecosystem. The COI sequences from several GSL taxa do not find exact matches in the barcoding databases, suggesting that this extreme ecosystem supports a surprising diversity of invertebrates.

Dietary versatility of coral reef fishes in response to habitat degradation

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Background: Dietary versatility represents a sensitive functional indicator towards environmental change effects in coral reef fishes. DNA-based approaches (metabarcoding) enable us to assess dietary composition at fine resolutions that were previously unattainable. Here, we compare metabarcoding of predator fish gut contents with observations of foraging behaviour (bite rates) and link this combined information to various states of habitat quality on the Mesoamerican Barrier Reef. This study refines our understanding of realized dietary niches for generalist feeding strategies crucial to conservation. **Results:** Metabarcoding of two invertebrate-feeding fish species (*Chaetodon capistratus*, a browser and *Halichoeres garnoti*, an active predator) revealed dietary responses for both species to variation in coral habitat quality. We used a combined primer approach using universal primer pairs targeting (*i*) a 313-bp region of the highly variable mitochondrial cytochrome *c* oxidase subunit I (mtCOI) gene, and (*ii*) 133- and 123-bp regions of the 18S nuclear ribosomal DNA (rDNA) gene to optimize taxonomic resolution and accuracy. We expect to find significant variation in dietary niche breadth in response to habitat degradation for both study species. This effect will be larger for the browsing species, indicating a behavioural switch from browsing towards active predation, thus implying fitness consequences of versatile feeding strategies when adjusting to degraded environments. **Significance:** We redefine generalist dietary spectra by revealing unexpected levels of dietary specialization. This indicates, against previous assumptions, that habitat degradation likely leads to suboptimal feeding conditions for fish species that opportunistically forage within the coral reef matrix. We further demonstrate that DNA-based gut content analysis allows for a fine-scale description of fish diets, surpassing conventional behavioural analysis, and show that combining both methods aids ecological understanding of critical mechanisms for the resilience of coral reefs.

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PhyloAlps: The genome project of the alpine flora

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Background: With the emergence of high-throughput sequencing technologies reducing the cost of sequencing by several orders of magnitude, we can imagine evolving the concept of DNA barcodes to exceed its current limits. For plants, classical DNA barcodes are often less performant than for other clades, leading scientists to consider to use more and more markers to resolve taxonomic ambiguities. Genome skimming, a global genomic approach at low cost, is now considered as an interesting alternative/complement to classical barcodes, but is it reasonable to imagine a complete switch to this technology? **Results:** The PhyloAlps project aims to skim the genome of each species of the alpine flora. This represents 6000 genome skimmings corresponding to about 4500 taxa or 4000 species. From these data, not all conventional DNA barcode sequences can be recovered, but most of the time, using the complete chloroplast genome, the complete nuclear rDNA cluster, and large parts of the mitochondrial DNA. After an enormous sampling effort, many developments at the bench and at the data analysis level, we have demonstrated that such an objective is doable, but we also touched the current limits of the technique, which are often linked to our limited biological knowledge, if we consider a complete flora and not only plant models. **Significance:** The PhyloAlps project, today replicated for the Arctico-Boreal flora, is the first of a long series of large-scale genome skimming projects targeting a complete and coherent set of species. Several similar projects targeting other flora over the globe are in the mind of scientists, in China, New Caledonia, and Spain to name a few, but also targeting animal groups like Collembola. With the continued decrease in cost of sequencing, genome skimming will soon provide much more than simple barcodes for less financial and technical efforts.

Deeply divergent COI lineages for the widespread Antarctic mite *Nanorchestes antarcticus*

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As part of the Antarctic Circumpolar Expedition (ACE) project *A Functional Biogeography of the Antarctic* (AFBA), we sampled individuals of the widespread mite *Nanorchestes antarcticus* from the Siple Coast in western Antarctica and compared them with previously obtained individuals from the Ross Sea Region. Specimens were obtained from the base of Mt. Siple and on nearby Lauft and Maher islands (73°S). These were compared with specimens collected from Mt. Kyffin and Mt. Harcourt near the Beardmore Glacier (83°S) as well as from the McMurdo Dry Valleys and Mt. Seuss on southern Victoria Land (77°S). All individuals were analysed at the COI gene locus (DNA barcode region). Maximum pairwise sequence divergences (>20%) were observed between individuals from Mt. Seuss near the Mackay Glacier and Mt. Harcourt near the Beardmore Glacier and between those from the Siple Coast and southern Victoria Land. Individuals collected from the Siple Coast were genetically most similar to individuals collected from the Beardmore Glacier sites albeit with >8% sequence divergence. In contrast, divergences within sites were generally <2%. We conclude that the highly fragmented landscape of present-day Antarctica, as well as repeated glacial cycles, has resulted in the deeply divergent lineages we observed. In some cases, these individuals are likely to have been isolated for several million years and may represent previously unknown species. Further work (e.g., eastern Antarctica) will likely reveal additional divergent lineages for this widespread species.

DNA barcoding of forensically important flies in the western Cape (South Africa): a pilot study

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Background: Forensic entomology provides a method to determine post mortem interval based on the age of and stage of life cycle of arthropods associated with a dead body. This requires knowledge of the life cycle of insects that visit the body, especially first colonisers such as Calliphoridae (Diptera). Traditional species identification has been hampered by morphological indistinguishability, especially between immature specimens or when specimens are damaged. Implementation of molecular methods such as DNA barcoding has introduced methods to complement morphological findings. However, in order to provide effective and correct identifications, databases need to be well represented. The utility of DNA barcoding for species-level identification was investigated using adult and immature species of blow fly common to the western Cape of South Africa, (*Chrysomya chloropyga*, *Chrysomya albiceps*, *Chrysomya marginalis*, and *Lucilia sericata*). The standard COI barcode, as well as a secondary barcode ITS2, was amplified and sequenced. **Results:** Sequence divergences within and between species were analysed. Intraspecific divergence showed a maximum of 0.003% and 0.043% for COI and ITS2, respectively. Higher interspecific divergence values were found in COI sequences compared to ITS2. DNA sequences from the adult specimens were then used as reference sequences for identification of seven unknown immature specimens using DNA barcoding of both COI and ITS2. Sequence similarity was assessed and identity was assigned based on >98% similarity scores, and all immatures were successfully identified. **Significance:** According to these results, COI and ITS2 have sufficient discriminatory power for species-level identification of the four species studied. Additionally, this technique of DNA barcoding is suitable for the identification of immature specimens.

Assessing DNA barcodes as a diagnostic tool for mosquitoes in nature and anthropized areas

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Background: Insects are a group of wide distribution and importance in the desert and semi-desert environments of Mexico. Therefore, a taxonomic distribution and diversity assessment of mosquitoes associated with mangroves and anthropic sites are required because of the medical importance of this group which is considered as a significant vector in Baja California Sur (BCS). Through DNA barcoding, mosquito species associated with mangroves and anthropic areas were defined. **Results:** This first approximation allowed generating the first reference library of DNA barcodes for mosquitoes in BCS (www.boldsystems.org/Arthropod and mangrove from Baja California Sur, Mexico AMBCS). The molecular tool was integrated into classical taxonomy to delimit mosquito species. Barcodes were obtained from 100 specimens representing 10 species (4% of the species present in Mexico and of medical importance). We obtained 85% of DNA sequences. The average percent of identification was >93%. Barcode Index Numbers (BINs) correspond to the limits of recognized species, which could be compared with the species present in mangroves versus anthropic localities. All individuals were correctly assigned to species (*Aedes aegypti*, *A. vexans*, *Culex coronator*, *C. pipiens*, *C. tarsalis*, *C. usquatus*, *Chironomus calligraphus*,

Psorophora columbiae, *Ochlerotatus taeniorhynchus*). **Significance:** This information is the baseline for the state health sector (SSABCS) and the first study that integrates taxonomic, ecological, and conservation information for BCS. Barcode reference information is compiled from mosquito species in the area, which is a fast and efficient tool for identification. We have collaborated with the health sector, providing previously unknown information that will relate to diseases transmitted by vectors, such as Dengue, Chikungunya, and Zika besides providing information that allows the conservation of habitats such as mangroves.

The African soil microbiology project

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The African Soil Microbiology Project aims to generate a low-resolution barcode survey of microbial diversity in soils across sub-Saharan Africa. The 3-year (2017–2019) project is funded by grants from USAID and the Oppenheimer Foundation. A consortium of researchers from universities across the African continent (currently including South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia, Kenya, Ethiopia, Cote d'Ivoire, Benin, and Tunisia) are undertaking a series of national soil sampling campaigns, for the recovery of surface soils at 50 km spacings from regional transects. The initial phylogenetic survey, using MiSeq sequencing of 16S amplicon sets, will target Bacteria and Archaea; ITS amplicon sequencing and full metagenome sequencing will be included in line with future funding. The primary objectives of this project are to establish a baseline survey of continental soil biodiversity and to correlate soil microbial species diversity with macroclimatic parameters. This project is a "first" for Africa, and it is concurrent with similar projects under development in Europe and North America.

Uses and misuses of environmental DNA in biodiversity and conservation

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Estimating biodiversity has been a central undertaking and a major challenge for biologists over the centuries. DNA-based species identification known as barcoding has firmly transformed the traditional approach to biodiversity science. The field is quickly transitioning from barcoding single individuals to metabarcoding complex communities of organisms often using noninvasive sampling of environmental DNA. This rapid evolution involves new sequencing technologies, bioinformatics pipelines, computational infrastructure, and experimental designs. All these changes require new, integrative, and coordinated approaches to identify species and interpret biodiversity estimates. In this dynamic research field, many studies based on environmental DNA remain insular; biodiversity estimates depend on the particular marker of choice, the quality of the DNA libraries, bioinformatics pipelines, and divergence thresholds implemented. The molecular operational taxonomic units (MOTUs) inferred are not easily recognizable across sites or studies, making inferences regarding species distributions or ecology less practical. The research community needs a reliable recognition system open to input, validation, and annotation from users. A coordinated advancement of DNA-based species identification that integrates taxonomic information and phylogenetic inferences with barcoding information would facilitate access to almost three centuries of taxonomic knowledge and one decade of building repository barcodes. Many conservation projects are time sensitive, research funding is becoming restricted, and informed decisions depend on our ability to apply an integrative approach to biodiversity science.

DNA barcoding of reef-associated fishes from India

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Background: India has a rich aquatic diversity spreading across different ecosystems. Coral reefs are unique with the highest fish diversity and provide nursery and breeding grounds to marine fishes. India has fringing reefs around the islands in the Gulf of Mannar, the Gulf of Kutch, and the Andaman and Nicobar Islands. However, these reefs are declining due to anthropogenic activities, and the fish diversity from these reefs has not yet been characterized using molecular markers. **Results:** In this study, DNA barcodes (cytochrome *c* oxidase subunit I (COI)) were developed for 50 species of fish representing Ambassidae, Bothidae, Coryphaenidae, Gobiidae, Lutjanidae, Labridae, Pomacentridae, Serranidae, and Tetraodontidae collected from the Gulf of Mannar, the Gulf of Kutch, and the Andaman Islands. The average genetic divergence values increased from lower (within species: 0.3%) to higher (between families: 21%) taxonomic relationships. Cryptic diversity was observed in species of Gobiidae, Serranidae, and Pomacentridae. Distance- and tree-based delimitation methods discriminated 97% of fish species with a sufficient barcode gap. **Significance:** The present results are useful for effective management of reef fishes. The barcodes will enrich the existing database and are useful for estimating phylogenetic diversity values of reef fishes for conservation purpose.

Widespread phylogenetic homogenization of plant communities from anthropogenic change

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Background: Biological communities are under anthropogenic pressures from widespread exotic species introductions and native extirpations. This often results in the mixing of formerly disparate biotas in a process referred to as biotic homogenization. Despite growing interest, attempts to investigate biotic homogenization have generally not incorporated phylogenetic information explicitly. Because phylogeny captures information on the evolutionary history of taxa, it provides a powerful tool for understanding ecosystem sustainability to ecological perturbations and for predicting future evolutionary shifts, as anthropogenic threats intensify. In this study, we used comprehensive phylogenies derived from DNA barcodes and explore evolutionary shifts of plant communities in mega-diverse floras of the world, to recent anthropogenic change from phylogenetic homogenization. **Results:** We demonstrate that current phylogenetic homogenization is geographically non-random with regard to introductions and extirpations. Regions experiencing high evolutionary shifts are driven largely by non-native introductions than by native species extirpations. **Significance:** The fact that most of these changes to biotic composition have occurred within only the last few hundred years is astonishing and points towards the kinds of changes we should anticipate in the coming century.

DNA barcoding the Galápagos flora to reveal the process of species assembly on an oceanic archipelago

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Background: Remote locations, such as oceanic islands, typically harbor relatively few species, some of which go on to generate endemic radiations. Species colonizing these locations tend to be a non-

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random subset from source communities, which is thought to reflect dispersal limitation. However, non-random colonization could also result from habitat filtering. We evaluate the imprints of these processes on the flora of the Galápagos islands using a barcode phylogeny of ~39 000 species alongside information on dispersal strategies and climatic suitability. **Results:** We show that the native Galápagos flora is a phylogenetically clustered subset of species from the South American mainland, with Ecuador, Peru, and Colombia being the most important species sources. However, contrary to expectations, we found that habitat filtering rather than dispersal limitation was the predominant process structuring plant species composition. **Significance:** We suggest that the standard assumption that plant communities in remote locations are primarily shaped by dispersal limitation deserves reconsideration. Our results might also help explain why adaptive radiation is common on oceanic archipelagoes—because colonizing species can be relatively poor dispersers with specific niche requirements.

Absence of cryptic species and population structure in *Lychnorhiza lucerna* (Cnidaria) from southwestern Atlantic Ocean

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Background: An important trait of planktonic species is the larval dispersal in a spatio-temporal context. Population connectivity of certain species can be achieved by long-lived oceanic larvae that can reach great distances and disperse through large areas. A group of specimens with great larval dispersal may have a larger connectivity and, consequently, the ability to re-establish after disturbances in certain regions. This scenario helps understand patterns of gene flow, distribution, and defining populations of jellyfish (Cnidaria) species around the world. The present study aims to verify cryptic species and gene flow of specimens of a large scyphozoan jellyfish, *Lychnorhiza lucerna*, from 12 different sampling points (hypothetical populations) along shorelines of Brazil and Argentina based on molecular markers (COI and ITS). **Results:** Based on obtained sequences, we could confirm that specimens of *L. lucerna* from different localities present a low genetic variability (COI - K2P 0.002 and ITS - K2P 0.003), indicating high connectivity and absence of cryptic species in this taxon. The specimens from more distant localities present at maximum three nucleotides of variation, a restricted genetic variability, therefore evidencing the high connectivity between specimens from different regions. This result indicates the existence of a metapopulation with few biogeographic restrictions. This situation can be alarming in case of species with possibilities of population explosion (jellyfish blooms) because this condition can extend to all small groups depending on the factor triggering such blooms. **Significance:** Global climate change is a key factor in understanding connectivity. Scenarios resulting from these changes can cause the global expansion of jellyfish populations, with the outcome of more connected populations and an even broader distribution of species as main consequences. A metapopulation pattern for a species with bloom potential would become dangerous for ecosystems and human affairs and they should be monitored.

The disconnect between fungal taxonomists and fungal ecologists using DNA barcodes: how can we bridge the divide?

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The increasing numbers of studies using DNA barcodes or environmental nucleic acid sequences (ENAS) to assess fungal diversity have exposed thousands of previously unknown, unculturable species. As a result, more than a third of fungal DNA sequences in GenBank are of environmental origin. But inconsistent annotation of these undescribed, sequence-based taxa limits functional access to the data. Consequently, these ENAS are rarely considered in other studies, especially not in taxonomic treatments. This problem is confounded by the fact that the *International Code of Nomenclature for Algae, Fungi, and Plants* at present prohibits the description of novel taxa known only from ENAS. Various options are being considered by the mycological community to amend the Code to allow the systematic nomenclatural treatment of these "orphan" taxa. One possibility would be to allow DNA sequences (ITS barcodes) as types instead of typical herbarium specimens or living cultures. As an example, a new species with a DNA sequence as type was recently described in a study based on two matching GenBank sequences of fungi inhabiting conifer wood, but that was generated in two independent studies. One came from an uncultured fungus clone from spruce in Sweden, and the other from cedar wood in Canada. The lineage containing these two sequences was phylogenetically different from related species and was described as "*Hawksworthiomyces sequentia* sp. nov. ENAS". It was suggested that the ENAS acronym should be used with the species name until a type specimen is found and designated, after which it can be omitted. Another option to label ENAS species is a numbering system, but it is unlikely that taxonomists will use numbered taxa in their studies. What is clear is that a decision is needed on how the Code should be amended to enable the naming of sequence-based taxa to facilitate their incorporation in other studies.

DNA barcoding approaches highlight conservation challenges in chikanda orchids in southern and southeastern Africa

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Background: Chikanda is a traditional dish made with wild-harvested ground orchid tubers belonging to three orchidioid genera, *Disa*, *Satyrium*, and *Habenaria*, all of which are CITES appendix II listed. Identification of collected orchid tubers is very difficult, and documentation of constituent species in prepared chikanda has hitherto been impossible. Here, amplicon metabarcoding was used in samples of six prepared chikanda cakes to study genetic sequence diversity and species diversity in this product. **Results:** Molecular operational taxonomic unit (MOTU) identification using similarity-matching reveals that species of all three genera were present in the chikanda samples studied. *Disa* was present in all of the samples, *Satyrium* in five of six samples, and *Habenaria* in one sample, as well as a number of other plants. **Significance:** The fact that each sample contained orchids and the presence of a wide variety of species from all genera in this traditional dish raise serious concerns about the sustainability of this trade and the future of wild orchid populations in the main harvest areas. This proof-of-concept study shows that IonTorrent PGM is a cost-effective scalable platform for metabarcoding using the relatively long nrITS1 and nrITS2 regions. Furthermore, nrITS metabarcoding can be successfully used for the detection of specific ingredients in a highly processed food product at genus level, making it a useful tool in the detection of possible conservation issues arising from commercialized trade or processed plant products.

DNA barcoding as a forensic tool against wildlife crime in southern Africa

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Background: Wildlife crime has continued to escalate in southern Africa due to the lucrative value of wildlife products on local and

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international black markets. The National Zoological Gardens of South Africa (NZG) is therefore involved in providing wildlife forensic services using DNA analysis for species level and (or) individual identifications to support crime prosecution for unregulated activities. These criminal activities include illegal wildlife hunting, poaching, possession, and trade in protected species. **Results:** The NZG has to date processed a total of 95 wildlife crime cases, with 69 of these requiring species identification of crime scene samples and confiscations from suspects. All species have been identified using the standard animal DNA barcode cytochrome oxidase I (COI) and the cytochrome *b* mitochondrial gene regions and a chain-of-custody species reference database. We have identified 42 different species from these illegal activities that include 30 IUCN Red List and legally regulated species on the South African Threatened or Protected Species (TOPS) list. This list also include CITES listed Appendix I and II species such as cheetah, elephant, leopard, lion, and pangolin that have international trade restrictions. Examples of some significant cases will be reviewed in this paper including some challenges that were encountered based on the state and condition of samples available for examination, reference sample collection, and storage and processing of cases. **Significance:** The effective use of DNA barcoding for species-level identification of endangered wildlife has contributed to the fight against illegal wildlife poaching and trade. The successful prosecutions resulting from some of these wildlife crimes indicate that genetic tools will assist with regulating wildlife crime to promote sustainable use for species conservation. By providing a rapid, reliable, and accurate forensic DNA analysis service, DNA barcoding has become a useful forensic tool in South Africa.

The ECOTROP field school: inventorying Afro-tropical invertebrate biodiversity through student activities and the use of DNA barcoding

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Tropical ecosystems have been popularized as the most biodiverse habitats on Earth. However, biodiversity research in the tropics has mainly focused on charismatic vertebrates and higher plants so far, neglecting invertebrates that represent the bulk of local species richness. As a consequence, our knowledge of tropical invertebrate communities remains strongly impeded by both Linnaean and Wallacean shortfalls, and identifying species in a study site often remains a formidable challenge that inhibits the use of these organisms as indicators for ecological and conservation studies. Here, we present a summary of the results of sampling activities conducted by students during the ECOTROP field-school, a training program in tropical ecology where African and European students gained training in fieldwork and study design, and became involved in the front-end processing of samples for DNA barcoding. Most of the activities were oriented towards local surveys of invertebrate biodiversity in forest and savannah ecosystems of the northern section of Lope National Park in Gabon. During five successive editions of the program, a total of more than 12 500 invertebrates were sampled, and more than 11 000 barcodes were generated. More data will be added in the near future through the processing of samples obtained from two Malaise traps deployed in a forest and a savannah for 12 months in 2014 and 2015. A total of nearly 3000 Barcode Index Numbers (BINs, as a proxy for species diversity) have been obtained to date, most of which belong to Lepidoptera (1664) and Coleoptera (709). For many groups of interest, the number of BINs observed exceeded the number of species recorded for the country. This highlights how combining standardized sampling, DNA barcoding, and experimental learning can significantly enhance local knowledge of biodiversity and ecological community dynamics, while training young biologists to meet the future challenges of biological conservation.

Species richness and biogeography of Lake Tanganyika estimated from environmental DNA metabarcoding

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Background: Lake Tanganyika is one of only a handful of ancient lakes greater than 1 million years old and is home to an astonishing 600 endemic freshwater species. About one third of these endemic species are cichlid fishes. Not unlike most freshwater resources around the world, Lake Tanganyika is undergoing major changes due in part to climate change and the growing human population of over 10 million people in the lake's basin. Our goals were to test the efficacy of environmental DNA methods to detect biodiversity from water samples ranging from the endemic vertebrate group of cichlid fishes to elusive and otherwise hard to study megafaunas such as hippos, water cobras, Nile crocodiles, and the spot necked otter. **Results:** From a transect along 280 km of coastline, a survey of 130 water samples revealed over 270 metazoan operational taxonomic units (OTUs) from the cytochrome oxidase I (COI) gene, spanning seven phyla (Annelida, Arthropoda, Chordata, Cnidaria, Mollusca, Nematoda, Platyhelminthes, Porifera, and Rotifera). Additionally, from a newly designed primer set specific to the family Cichlidae, we detected over 100 OTUs from 32 genera of cichlid fishes. The eDNA detection of cichlid biodiversity is potentially correlated with rock versus sand habitat and suggests some transport of eDNA in the lake along the coastline. **Significance:** We demonstrate that eDNA metabarcoding of water samples is a powerful tool to rapidly generate knowledge about biodiversity and potentially their distributions in Lake Tanganyika, ecological data that can be used for management decisions for this large and endemic freshwater fauna.

DNA barcode library for North American butterflies

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Background: There is a widely recognized need for comprehensive knowledge of biodiversity, and DNA barcoding has emerged as the tool that makes this possible. Although butterflies are the most-studied group of invertebrates and possess a well-established taxonomy, recent barcode studies have exposed discordances with the current classification. **Results:** Our work is compiling a DNA barcode reference library for all North American butterflies. It currently includes 18 000 records from 750 species, providing an average of 20× coverage for 90% of the fauna. This presentation will review the performance of DNA barcoding and use barcode data to test current taxonomy. While the mean intraspecific p-distance was 0.5%, the mean nearest neighbour p-distance was 7%. Most (85%) species were

monophyletic, but the others showed evidence of paraphyly or polyphyly. A comparison between Barcode Index Numbers (BINs) and species recognized by current taxonomy revealed a perfect match for 70% of the species, 12% involved a merge of two or more species, and 18% were split in two or more BINs. **Significance:** By generating one of the first continental-scale DNA barcode libraries for an entire taxonomic group, this study has created a resource that facilitates the identification of North American butterflies regardless of life stage or specimen quality. As well, it provides a basis for improvement of the taxonomic system by assessing the incidence of potential cryptic and over-split diversity.

Rainforest tree composition and regeneration investigated using DNA barcoding in the Lower Kinabatangan Floodplain, Sabah, Malaysian Borneo

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Background: The Lower Kinabatangan Floodplain in Sabah, Malaysian Borneo, contains a partially fragmented rainforest corridor, varying in width on either side of the Kinabatangan River. It contains a range of secondary forest habitats and supports an extremely high level of biodiversity. Extensive oil palm plantations surround the forest, and in some areas native trees have been replanted in order to create a continuous rainforest canopy. We are interested in the resilience of the forest to reductions in size, increased fragmentation, and changes in the composition of animals involved in pollination and dispersal. A DNA barcode library of adult trees is being created and used to identify saplings in order to investigate forest regeneration. We are also assessing the feasibility of using DNA metabarcoding to conduct rapid biodiversity assessments of the rainforest understory. **Results:** We have sampled 10 permanent plots (each 50 m^2) within established forest and two plots within replanted regeneration sites. In total, 385 adult trees and 872 saplings have been DNA barcoded with *rbcL*, *matK*, *trnL*, and ITS2. A biodiversity sweep, consisting of collecting small leaf samples of every sapling for DNA metabarcoding has been conducted for eight plots. We will compare richness, diversity, and phylogenetic composition of adult and sapling communities within secondary forest habitats and the planted regeneration sites. **Significance:** Rainforest destruction continues to accelerate with significant losses of biodiversity and its associated ecosystem services. In Sabah, intact forest within protected areas covers only 8% of the land surface. As primary forest has been lost, secondary forests are now assuming greater importance for biodiversity. There is an urgent need for increased understanding of natural regeneration within these forests and robust protocols for replanting. DNA barcoding allows rapid identification for biodiversity assessment, monitoring, and conservation planning.

Developing an optimal strategy for agricultural bio-surveillance using DNA barcoding

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A recent census of agriculture conducted by Statistics Canada indicated that the national agricultural sector had an annual value of $51 billion. By providing an augmented capacity to discriminate species that attack or otherwise affect crops, DNA barcoding helps to secure the productivity of this sector. Consequently, several federal agencies in Canada now employ DNA barcoding as a routine tool in pest species diagnoses, but this approach has been implemented on a specimen-by-specimen basis. The recent development of metabarcoding and high-throughput sequencing (HTS) protocols create the potential to establish monitoring programs that deliver near real-time information on the presence and absence of insect pests, information that farmers could employ to optimize the timing, dosing, and nature of pesticides or biological control agents used to protect their crops. The Centre for Biodiversity Genomics is developing an agricultural monitoring protocol by establishing a preliminary species inventory of arthropods found in common Canadian crop fields, assessing various factors related to site sampling, and obtaining results in a rapid manner by employing HTS protocols. In the first year of this project, Malaise traps were deployed at two research farms near Guelph, Ontario, covering six fields and four crop types, with variable trap orientation and placement. Weekly trap samples underwent lysis, DNA extraction and COI amplification in bulk, and products were then pooled prior to sequencing on an Ion Torrent S5 system. Sequence data processing and analysis were executed using the new mBRAVE platform. Preliminary results were promising in terms of feasibility and scalability, and set the stage for future work where sampling can be expanded to numerous sites and crop types across the country, and where turnaround time from sample to report can be dramatically reduced.

DNA barcoding resolves the highly complex banana sub-species and synonyms

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Background: The banana (*Musa* sp., AAA) genome is continuously expanding due to the high frequency of somaclonal variations. Due to the large diversity, a conventional numerical and morphological method of taxonomic and phylogenetic based identification of banana cultivars is laborious, difficult, and often leads to the subject of disagreements on commonly used synonyms that determines the market value. **Results:** The ITS2 region was used for identification and to find the genetic relationship between the cultivars and varieties of banana. Sixteen banana samples were locally collected and PCR amplified. Along with this, 321 sequences were retrieved from GenBank, USA, and used in this study. The sequences were aligned using Clustal W, and genetic distances were computed using MEGA V5.1. There was a significant divergence between the intra- and infraspecific genetic distances of the ITS2 region; the presence of a barcoding gap was obvious. BLAST1 and distance methods results showed that the ITS2 region possesses 97.7% and 95.8% identification success rates at species level for 345 samples of *Musa* using BLAST1 and nearest genetic distance, respectively, and could successfully identify and distinguish the cultivar and varieties of banana. As well, in this study, ITS2 revealed the relationship between cultivar and varieties of banana. **Significance:** ITS2 has been proven not only as an efficient barcode to identify the banana species but also as the potential candidate to study phylogenetic relationships between the sub-species and cultivars. Hence, this is the first comprehensive study on banana sub-species and varieties using DNA barcodes. Thus, ITS2 provides a better understanding of the origin and domestication of cultivated banana. In this regard, we have studied the molecular phylogeny and nucleotide diversity among the domesticated banana plants, which gave the information on parental and maternal donors of the studied cultivars and more clarity on commonly used synonyms that determines the market value.

First DNA barcode library of fishes from biodiversity hotspot Lower Paraná River in Argentina

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Background: The Lower Paraná River floodplain comprises one of the largest and biodiverse wetland systems of South America. More than

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164 freshwater fish species have been described in the lower Paraná River, but most lack associated molecular information and many lack barcode reference sequences available in the Barcode of Life Data System (BOLD). The present study aims to assemble a comprehensive reference sequence library for fishes of the lower Paraná River and to evaluate DNA barcodes' effectiveness for their identification. **Results:** Taxonomic identification of the 410 fish specimens resulted in 90 species (70 genera, 31 families, and 10 orders) from the lower Paraná River. The average K2P genetic distance between specimens was 0.4% within species, 12.64% within genera, and 20.67% within families. The average divergence within conspecific specimens was 24-fold lower than the average found in congeneric species, evidencing the existence of a "barcode gap" that allowed unambiguous discrimination of 97% of species. Interspecific genetic distances ranged from 4.06% to 22.56% (average of 14.56%), with the exception of two species of the genus *Odontesthes* in which members have minimum genetic distance of 0%. In addition, four species showed deep intraspecific divergence (>2%): *Hoplias malabaricus* (7.59%), *Brycon orbignyanus* (6.68%), *Potamotrygon motoro* (3.32%), and *Cnesterodon decemmaculatus* (3.16%). **Significance:** A reference barcode sequence library of fishes of the Lower Paraná River is presented for the first time for future use in identification of these species, and allowing them to be available for use in other applications. Five new records were generated and submitted to BOLD that had not been studied (*Parastegophilus maculatus*, *Pseudohemiodon laticeps*, *Magalonema argentinum*, *Auchenipterus nigripinnis*, and *Xyliphius* sp.). In addition, the review of the individuals whose groups presented deep intraspecific divergence confirmed the presence of more than one species in each group.

Africa, freshwater, and dragonflies: natural history and conservation in the continent of change

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Freshwater conservation is not just another challenge but embodies all environmental problems: as we disrupt the cycles that ensure its flow, we cannot expect one source to provide a sewer and a drink, energy, and irrigation, and still sustain life forever. Dragonflies and damselflies are popular indicators of aquatic integrity and among its most powerful symbols. Found only but in all freshwater, they represent the majority of aquatic life, insects. As conspicuous members of a neglected majority, they emerge from a forgotten world into our lives on land. And, on the wing, they can return as quickly when things improve as they vanished when it got bad. Capturing nature's beauty, needs, and resilience, dragonflies can become for healthy rivers and clean water what bees are for pollinators and food security. I will discuss how the growing interest, together with methodological innovations, can change the image and assessment of our most valuable environments. I focus on Africa, perhaps the most changeable continent historically, but definitely where man-made change will be most dramatic this century.

A high-resolution genetic map of European butterflies provides a unique resource for research and nature conservation

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Background: The European butterfly fauna is arguably the most intensively studied invertebrate group in the world and represents a key resource in providing models for research and nature conservation. Therefore, accurate knowledge of butterfly species composition and distribution, as well as their genetic features, has numerous theoretical and practical implications. Since time and material resources are limited, wide-scale surveys based on DNA barcodes are particularly useful because they highlight patterns of notable interest that can be later investigated in a more detailed manner. Previous research using DNA barcodes highlighted complex biogeographic patterns and unexpected levels of potential cryptic diversity for some European regions, but a comprehensive overview of the continent's butterfly fauna at high spatial resolution is lacking. **Results:** Within the framework of the EUGENMAP project, we assembled a high-quality, continental-scale DNA barcode library for European butterflies, which comprises 20 000 DNA sequences representative for 99% (460 species) of the continent's butterfly fauna. Ongoing analyses of this reference library allows us to assess patterns of genetic diversity, which help explain the phylogeography of numerous species and prioritize conservation efforts. Furthermore, we detect a series of cases involving either deep intraspecific divergence or DNA barcode sharing with one or several species, thus highlighting potential gaps in knowledge and the need for further research. Using a series of selected examples, we show what insights into the evolutionary history of organisms can be obtained when DNA barcodes are combined with additional data. **Significance:** Europe is the first continent to benefit from a comprehensive DNA barcode library for its butterfly fauna. This library represents a valuable resource that will likely foster research in butterfly taxonomy, ecology, and evolution, as well as practical applications in specimen identification and conservation.

Combined analysis of morphological and genetic markers reveals five probable species in the widespread taxon *Khaya anthotheca* (Welw.) C.DC (Meliaceae)

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Background: The delimitation of cryptic species is a major challenge for taxonomists because it has implications not only for the interpretation of phylogenies and the understanding of evolutionary processes but also for the management and conservation of biodiversity. Most descriptions of species are consistent with what can be considered the concept of morphological species attached to a type. However, speciation is not always accompanied by sufficient divergence in morphological characteristics for the clear separation of species. Occasionally, what is considered a single species on morphological grounds is likely to contain a complex of species on biological bases. This is the case of *Khaya anthotheca*, one of the African mahoganies, of the family Meliaceae. Due to its morphological plasticity, the delimitation of this taxon varies according to the author. By combining a morphological study of herbarium specimens with the genotyping of hundreds of samples using nuclear genetic markers (SNPs), we propose a solution to this problem. **Results:** Nuclear SNPs allowed us to distinguish five different genetic groups. Three of these five groups have allopatric or parapatric distributions and two of them are locally sympatric. Recognition of these genetic groups was reinforced by a fine analysis of morphological characters so that they should be con-

sidered as separate species. **Significance:** These results are particularly important given the pressure of logging on African mahoganies, as some of these groups may correspond to cryptic species that could be threatened by overexploitation.

Growth and ecological requirements of *Crassocephalum crepidioides* (Benth.) S. Moore for the production in nurseries

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This research focuses on the germination, growth, and ecological requirements for the production of *Crassocephalum crepidioides*, a species native to tropical Africa and Madagascar. The objective is to investigate the conditions in nursery production of the species, in the aim of market gardening diversification and the struggle against food insecurity. A seed germination test has been carried out with 1800 seeds of *C. crepidioides* on traditional seedbeds. Pricking out of the plants was carried out on soils fertilized with the dung of cows and droppings of poultry. Additional samples without fertilizers were used in order to study the effect of soil fertilization on the growth of plants. These plants have been exposed differently to light (out of shelter and under shelter) in order to determine the effect of sunstroke on the growth of *C. crepidioides*. These two studied factors enabled us to determine some conditions of the production of this plant as a vegetable. Results showed that the seeds of *C. crepidioides* have a latency time of ~18 days and a germination rate of 15%. Fertilizing with the dung of cows and droppings of poultry is appropriated globally for the cultivation of species. Five weeks after pricking out, the mean values of growth parameters on dung of cows and droppings of poultry are, respectively, as follows: total height 22.85 and 23.33 cm; number of leaves 8.60 and 7.77; length of leaves 19.90 and 20.52 cm; width of leaves 7.19 and 7.24 cm. Under the shelter and out of shelter, the mean values of growth parameters are, respectively, as follows: total height 23.70 and 14.75 cm; number of leaves 18 and 22.50; length of leaves 19.28 and 16.45 cm; width of leaves 7.04 and 5.82 cm.

Using next generation and modified PCR approaches to DNA barcode species of *Drosophila* and their parasitoid wasps in the eastern USA

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The goal of the project for which we developed these methods is to investigate host–parasitoid trophic relationships among *Drosophila* flies and their parasitoid *Leptopilina* wasps in fruit orchards in the eastern United States. Ultimately the project will provide information on species richness, relative abundance of hosts and parasitoids, and species associations, including specificity and frequency. We had difficulty barcoding many specimens of *Leptopilina* wasps using traditional COI barcoding methods due, we hypothesize, to co-amplification of remnant *Drosophila* host DNA along with the wasp DNA. PCR products of these mixed COI sequences could not be cleanly sequenced using Sanger methods. We therefore tested methods for teasing apart the DNA sequences of host and parasitoid. We designed primers for a short variable region of 28S that amplifies in both taxa. Using these primers we amplified the region from parasitized *Drosophila* pupae and empty pupal cases and developed next-gen libraries from the amplification products. Adapters and sample-specific indexes were ligated to each amplification product, and equal amounts of DNA from each was then run on a nextgen platform. Sequences from each indexed sample were compared to reference sequences for both fly and wasp. We were able to recover sufficient sequences from each sample so that both fly and parasitoid could be identified. Next steps include optimizing the method using the traditional barcode marker COI. In addition, we developed a method of PCR using blocking primers that allowed for direct amplification and Sanger sequencing of the barcode region from both fly and wasp. We developed two sets of blocking primers: one to block the amplification of fly DNA so that the wasp could be cleanly amplified, and the reciprocal set. Using two PCR reactions with these sets of primers for each pupal specimen, we successfully amplified both host and parasitoid COI.

High spatial and temporal turnover in the arthropod community of a tropical montane forest

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Background: Although spatial turnover in community composition is generally thought to be greater at low than high latitudes, few studies have evaluated this pattern for terrestrial arthropods, and fewer still have examined how community composition changes with time. The present study addresses this gap by examining the spatio-temporal patterning of terrestrial arthropod communities at two sites, roughly 2 km apart with a 400 m elevational difference in a tropical montane forest in Honduras based on Malaise trap sampling from June to August in four consecutive years (2012–2015). **Results:** Despite the analysis of 24 752 specimens, only 9%–18% of Barcode Index Numbers (BINs) at a site were shared between years, and only 10% of 5006 BINs were shared between sites. Even when sampling was intensified in 2015 with the use of three traps per site and 47 326 specimens analysed, the proportion of shared BINs did not increase. In total, 6682 BINs were collected at the two sites with the low-elevation site being more species rich (4943 vs. 2493 BINs). Although positioned just 5 m apart, only 14% of BINs were captured by all three traps at a site, and more than 60% were unique to one trap. Overall, temporal variation contributed less than spatial variation to the differentiation of each community, but it was more pronounced when analyzed at a species than a family level. **Significance:** These results highlight the large sample sizes needed to adequately characterize biodiversity patterns in tropical ecosystems. They also establish the important role of standardized approaches for arthropod sampling and species identification in facilitating large-scale analysis. Finally, they support the conclusion that enhanced spatio-temporal variation contributes to the latitudinal gradient in species richness. Further exploration into baseline rates of species turnover in varied settings is essential to advance understanding of the processes that underpin community assembly and to better manage habitats at risk.

Species gap analysis in DNA barcode reference libraries of macrobenthic fauna from transition and coastal waters along the western European Atlantic coast

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Background: Comprehensive and reliable barcode reference libraries are essential elements of any DNA-based monitoring tool. Ideally, reference libraries should cover the full sweep of species in the target ecosystem, with a balanced geographic representation of each species distribution range. It is therefore essential to assess the missing taxa and geographic breadth of the existing records. We conducted a species gap analysis for the most prominent groups of marine benthic invertebrates that are relevant for the biomonitoring of coastal eco-

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systems in Atlantic Europe. **Results:** The synonym-filtered checklist comprised 2525 species (1055 Annelida, 853 Crustacea, and 617 Mollusca), and the gap-analysis of mitochondrial cytochrome *c* oxidase subunit I (COI) barcodes was conducted in BOLD. The percentage of species missing COI barcodes was above 50% for all the targeted groups (51% Crustacea, 56% Mollusca, and 59% Annelida). All classes of Crustacea and Annelida in the checklist were represented by COI barcodes, but for Mollusca representative taxa were missing barcodes for two classes out of six. Annelida and Crustacea lack representative barcodes for more than ~6% of the orders, and up to ~30% of the molluscan orders were also missing. At family level, the gap was between 15% (Annelida) and 20% (Crustacea and Mollusca). **Significance:** Although the targeted species belong to dominant groups occurring in Atlantic European coasts, current reference barcode libraries are still lacking a fair proportion of relevant species and higher taxonomic levels. This study indicates where to prioritize efforts to complete the reference libraries, starting from the higher taxonomic ranks down to species level for these three large taxonomic groups, but efforts must also be extended to other groups if not yet conducted (e.g., Echinodermata, Nemertea, Bryozoa). This work was developed in the scope of a short-term scientific mission of the COST Action CA15219 - DNAqua-Net.

DNA barcode diversity of freshwater calanoid copepods (Crustacea) from New Zealand and Australia infer recent dispersal and local evolution of the New Zealand fauna

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Background: We examined DNA barcode diversity (COI, 28S) for calanoid copepods from New Zealand and Australia, and we tested the hypotheses that these data would support existing models of speciation by vicariance for Australasian freshwater representatives of the family Centropagidae. **Results:** COI sequence divergences between Australia and New Zealand for species of *Boeckella* and *Calamoecia* were <19%. This implies that species in New Zealand diverged from Australia in the last 10–15 MYR, suggesting dispersal much later than the 80 MYR needed to support vicariance as the mechanism of speciation. New Zealand and Australian species of *Boeckella* were found to group together genetically, and both were separated from representatives from South America, which split from Antarctica in the last 30-50 MYR. Further, endemic species of *Boeckella* from Australia and New Zealand were found to be closely grouped (e.g., *B. montana* and *B. tanea*, respectively), indicating recent dispersal. **Significance:** We conclude that extant New Zealand calanoid copepod taxa are the result of dispersal since the Oligocene transgression (24 MYR) and not vicariance following the breakup of Gondwana. These findings are thus incongruent with models suggesting a more ancient origin of the Centropagidae, which had been based on the distributions of extant genera.

Progress and prospects of the Norwegian Barcode of Life Network (NorBOL)

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For 10 years, the Norwegian Barcode of Life Network (NorBOL) (www.norbol.org) has been the national network for DNA barcoding of the fauna, flora, and fungi of Norway. The network is going strong due to active partners and funding from the Research Council of Norway and the Norwegian Biodiversity Information Centre. NorBOL is coordinated by the NTNU University Museum in Trondheim and connects 17 institutions, including all four major natural history museums as well as all major biological research institutes in Norway. The initiative uses the Barcode of Life Data System (BOLD) as a repository, which currently holds more than 70 000 published sequence records (45 000 DNA barcodes) of more than 14 000 species from Norway. Our goal is to barcode 20 000 species from Norway and Polar regions by the end of 2018. NorBOL currently targets barcoding of terrestrial arthropods, plants, lichens, fungi, marine invertebrates, fish, and material from bioinventory projects supported by the Norwegian Taxonomy Initiative. We collaborate with Swedish and Danish institutions on sampling of marine invertebrates, earthworms, and terrestrial arthropods and are engaged in low coverage shotgun sequencing of herbarium material to obtain full barcodes of vascular plants. As the barcode library for Norwegian species grows, applied projects that use the national research infrastructure have emerged. The number of BOLD users from Norway increases steadily. In 2016, users from Norway generated almost 2 million hits from about 7000 unique IP-addresses. There are currently 138 entries (publications and other activities) linked to NorBOL in the National Research Information System CRIStin. We expect further increase in activity as NorBOL moves forward and especially welcome collaboration on DNA barcoding of Polar biodiversity.

Applying DNA metabarcoding for routine stream monitoring and beyond

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Background: Recurrent assessments of freshwater macrozoobenthic diversity are widely applied to monitor stream health. Unfortunately, most taxa are sampled at larval stage and can often not be accurately identified based on morphology. Additionally, identification can be time-consuming. Thus, the approach leads to high assessment costs while providing limited accuracy and resolution. Here, DNA metabarcoding has emerged as a potential solution, but biases of the technique are currently not sufficiently evaluated to allow application in routine assessments. Two main biases of metabarcoding are (*i*) effects of different taxon biomass in complete bulk samples and (*ii*) primer bias leading to the over- or underrepresentation of taxa, thus negatively influencing assessment accuracy. **Results:** Using 10 mock bulk communities each containing 52 different freshwater taxa and primer evaluation tools (PrimerMiner) we tested and developed potential solutions for these known biases. Eight different primer pairs were investigated using DNA metabarcoding and a primer pair suited for macroinvertebrate detection identified that shows only little primer bias and high detection rates (BF2+BR2). Using our optimised DNA metabarcoding protocols, we processed 18 bulk samples from routine monitoring of Finnish streams. With DNA metabarcoding, we detected on average 57% more taxa than morphological inspections, and DNA-based assessment indices were very similar to morphology-based indices. Nevertheless, we identified priority areas for further optimisation, e.g., reference databases and centralised cloud-based bioinformatics solutions. Finally, first results of pioneering studies using metagenomic approaches and haplotype-based metabarcoding are presented. **Significance:** Using the data from the Finnish stream monitoring program we show that DNA metabarcoding can detect most taxa present in a ecosystem and is already reliable and reproducible enough to be used in routine assessment of freshwater Macrozoobenthos. We, however, also stress that DNA metabarcoding protocols have to be carefully validated and optimised for ecosystems and taxa of interest.

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Improved protocols to accelerate the assembly of DNA barcode reference libraries for freshwater zooplankton

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Background: The recovery of DNA barcodes from freshwater zooplankton has been challenging. As a result, most records in the Barcode of Life Data System (BOLD) for the three main groups (Rotifera, Cladocera, and Copepoda) derive from Canada and (or) Mexico, with the exception of rotifers, where New Zealand is playing an important role. **Results:** This study demonstrates that improved fixation protocols involving the use of ice-chilled ethanol, coupled with the use of zooplankton-specific primers (Zplank), greatly improves success. We also used novel collecting methods involving light traps and plankton tows in several Canadian and Mexican lakes. Using these protocols to analyze taxonomically diverse assemblages (rotifers, mollusks, mites, crustaceans, insects, and fishes), we recovered sequences from 85% of 2136 specimens. The analysis of 922 specimens from Bacalar Lake, a karstic oligotrophic tropical system in Mexico, revealed 84 Barcode Index Numbers (BINs), with almost half detected in a single light trap sample. Many of these species were newly detected, including several mysids, sesarmids, and amphipods of marine origin. Additionally, the analysis revealed that Lake Bacalar is a nursery area for the early stages of a marine fish. A brief sampling effort of two nights with a light trap in a Canadian lake revealed 87 BINs, including 2 rotifers, 26 cladocerans, 16 copepods, 4 ostracods, 15 mites, 23 insects, and 1 fish. Finally, from a total of 325 BINs, only two cladocerans and one copepod are shared between Mexico and Canada, suggesting a smaller geographical distribution than previously thought for freshwater zooplankton. **Significance:** These protocols will help to assemble comprehensive DNA barcode reference libraries, facilitating programs that monitor ecosystem health and track invasive species. This will open a new window for conservation while also advancing understanding of the ecology and distribution of freshwater zooplankton.

The potential for rapid, local DNA barcoding using the Oxford Nanopore Technologies MinION to expedite the processing of wildlife forensic samples and the prosecution of perpetrators

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Background: Wildlife crime represents not only a threat to biodiversity in environmentally vulnerable areas of the globe but also to the economies of developing nations and safety of their citizens, as poaching and trafficking frequently funds organised crime and terrorism. **Results:** We have developed a workflow, which takes advantage of both rapid lysis and fast, portable sequence generation using the Oxford Nanopore Technologies MinION™ sequencing platform. The MinION™, combined with a portable laboratory, allows for DNA to be extracted and PCR amplified and prepared into a single NGS library from up to 20 tissue samples. This library is sequenced using the small, portable MinION™ nanopore sequencer, the reads of which are processed locally on a sequencing laptop and then uploaded to mBRAVE, our online Barcode of Life Data System (BOLD)-connected metabarcoding analysis platform. Our workflow can proceed from tissue sample to barcode sequence in under 10 h. Verified reference libraries from BOLD can ensure accurate assignment of samples to species, and by using PCR barcoding Barcode Index Numbers (BINs) from different samples can be easily differentiated from one another. **Significance:** The ability to proceed from sample to species ID rapidly will facilitate the rapid, on-site enforcement of laws and prosecution of traffickers and poachers, and preserve threatened biodiversity in developing nations.

Barcoding African freshwater sponges holotypes and the Sponge Barcoding Database v2.0

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The Sponge Barcoding Project (www.spongebarcoding.org) aims to facilitate unambiguous species identification for sponges on grounds of the standard barcoding and other molecular markers. More than 10 years after its foundation, the Sponge Barcoding Project continues to establish a reference backbone database. African sponges, particularly freshwater sponges, are largely understudied, compared to demosponges in most other geographical regions. Recent morphological studies started summarizing our current knowledge and conclude that the species richness of African freshwater sponges is underestimated and that geographic distribution ranges remain obscure. Freshwater sponges (*Spongillina*) likely share a common ancestor; however, their evolution, particularly the radiation into endemic and allegedly cosmopolitan groups, is largely unknown. Freshwater sponges of at least 58 species of 17 genera and at least four families are described from Central and Eastern Africa, but the diversity may be largely underestimated, owing to limited distinguishable morphological features. The discovery of additional, particularly cryptic, species is very likely with the use of molecular techniques such as DNA barcoding. The Royal Museum of Central Africa (RMCA, Tervuren, Belgium) hosts one of the largest collections of (Central) African freshwater sponges and their type material. Holotypes, in theory, constitute ideal targets for molecular taxonomy; however, the success is frequently hampered by DNA degradation and deamination, which are a consequence of suboptimal preservation techniques. Therefore, we genotyped African demosponge holotype material of the RMCA with specific short primers suitable for degenerated tissue and compare the results with the current, morphology-based classification. Our results will be presented combined with the presentation of the new user interface of the Sponge Barcoding Database. The new version will comprise important new and updated features, tools, and links, making molecular sponge identification much more accessible to the non-taxonomist.

Large-scale assessment of COI adaptation to high altitude in birds

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Background: Adaptation to hypoxic highlands has been widely studied in diverse organisms, mainly by analyzing genetic and amino acidic changes in haemoglobin and their physiological consequences. In spite of the relevant role of mitochondrial genes in the cellular respiratory process, their adaptation to high altitude has been less studied. Moreover, contrasting results have been found, with apparent adaptation present only in some high-altitude species. Broad analyses are therefore needed to establish general patterns of mitochondrial adaptation to hypoxic environments. In this context, and taking advantage of the large-scale genetic library generated by the Barcode of Life project, we studied COI adaptation to high altitude in birds in the Americas. **Results:** Over 22 000 COI sequences from around 2000 avian species from the American Continent were retrieved from the Barcode of Life Data System (BOLD). Using a complete phylogeny of the birds of the World, we classified 155 pairs of sister species into highland–lowland, highland—highland, and lowland–lowland species pairs to compare their COI sequences (2300 sequences used in total). Even though we did not find evidence of a generalized adaptation to high altitude in COI, there was a tendency towards more changes in amino acids and a higher proportion of sister species with differences in their amino acids in highland–lowland and highland–highland species pairs than in lowland–lowland species pairs. We also analyzed the amino acids that did differ between highland and lowland species to assess their position and whether their properties

differed to assess whether the modification could affect the protein structure and function. **Significance:** This is the first large-scale analysis of mitochondrial adaptation to high altitude in any taxonomic group, and the results suggest that the adaptation of COI to hypoxic highlands in birds is idiosyncratic. This study constitutes an example of how DNA barcode libraries allow diverse large-scale analyses beyond species identification and discovery.

DNA barcoding supports morphological evidence for a new genus of Alcyoniidae (Cnidaria: Octocorallia)

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Background: Alcyonacea are conspicuous members of reef communities, yet the study of their ecology is confounded by inconclusive taxonomic knowledge. The addition of phylogenetic analyses and DNA barcoding to traditional taxonomic approaches has progressed our understanding of genus and species boundaries; however, few markers are suitable for studies of Alcyonacea as they are predominantly mitochondrial and evolve significantly slower in Octocorals than their nuclear counterparts. We investigated the taxonomic resolution of DNA barcoding in a number of shallow-water genera from the Indo-Pacific and assessed the utility of a nuclear ribosomal gene (28S rDNA) in addition to known mitochondrial markers. **Results:** We found that a mitochondrial and nuclear multi-locus barcode (2300 bp), consisting of COI+igr1+MutS+28SrDNA, revealed clades with strong support to species level. We especially targeted one genus, where morphological evidence indicates that a number of species should probably be reassigned to a new genus, and found it was substantiated by a highly supported monophyletic clade distinct from other alcyoniid genera. In addition, 28S rDNA exhibited more parsimony informative sites, higher haplotype diversity, and delineated between species, making it a powerful addition to the existing mitochondrial barcode. **Significance:** Here, we present phylogenetic evidence for a proposed new genus and the first DNA barcodes for these taxa. This supports the reassignment of those species found to exhibit morphological discrepancies with their designated genus and incorporates incipient morphospecies. The biogeographical distribution of this proposed new genus currently includes reefs of South Africa, Mozambique, Mauritius, Seychelles, the Nicobars, Fiji, Australia, and American Samoa.

Assessing freshwater biodiversity in the Malay Archipelago: an eDNA approach

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Background: The use of environmental DNA (eDNA) metabarcoding to detect eukaryotic organisms has exploded in recent years, with study topics ranging from Vietnamese mammal distribution found from leech blood to bee foraging activity found from honey. Combining conventional barcoding approaches with next-generation sequencing technologies allows a massive amount of biodiversity information to be generated. An often-overlooked reservoir of biodiversity is found in freshwater ecosystems, which constitute 0.01% of Earth's water, but they are estimated to contain roughly 9% of all described species. Southeast Asia hosts the highest threatened freshwater species richness in the world, where habitat degradation, pollution, and climate change impact biodiversity. This project aims to assess freshwater biodiversity from Malaysian and Indonesian lakes, with relation to water chemistry, habitat quality, and biogeography through filtering of water to extract eDNA. **Results:** Final results have not yet been generated, but initial data is as follows. Samples were collected in triplicates along transects of lakes from Bali, Sulawesi, Kalimantan, Sumatra, Java, and Peninsular Malaysia. Three genes were targeted to maximise the amount of biodiversity information generated. PCRs were set up in DNA-free conditions, and tagged primers were used with a twin tag set up. Initial data showed that (*i*) COI = 26 243 410 reads and 15 766 484 unique sequences, (*ii*) 12S = 25 180 573 reads and 22 470 959 unique sequences, and (*iii*) 16S = 23 518 192 reads and 15 171 779 unique sequences. Samples were pooled for sequencing using Illumina MiSeq. **Significance:** As the Malay Archipelago contains the World's highest threatened freshwater species richness, conservation measures must be improved to protect these vulnerable ecosystems. The metabarcoding data from this project will evaluate the use of eDNA as a cost effective and reliable tool for assessing biodiversity in this region and make conclusions regarding the level of habitat disturbance in relation to the amount of biodiversity observed.

Surveying estuarine meiofauna through DNA metabarcoding: optimization of sampling and molecular protocols

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Background: DNA metabarcoding made possible the high-throughput profiling of biological communities, especially those difficult to track, such as marine meiofauna. However, considerable improvement and optimization of metabarcoding protocols are still required. Here, we studied both (*i*) the amount of sediment sample used for environmental DNA (eDNA) extraction and (*ii*) the target region and primer-pairs used for PCR amplification, and their impact on taxonomic profiling of estuarine meiofauna. For this, eDNA was extracted from intertidal sediments (0.63, 2.50, and 10.0 g samples) collected in the Lima estuary, Portugal. Five PCR amplifications were obtained by targeting V1-V2 (~400 bp), V4 (~400 bp), and V9 (~250 bp) of the 18S rRNA gene, and two internal regions of the COI barcode, here identified as COI-A (~313 bp) and COI-B (~310 bp). Then, amplicons were sequenced in an Illumina-MiSeq platform. **Results:** Reads were processed using customized procedures in mothur, operational taxonomic units (OTUs)-clustered at 97% similarity, and subsequently BLASTed against SILVA (ribosomal RNA) and BOLD (COI) databases. Resulting rarefaction curves approached saturation for all primers and amounts of sediment tested, while the highest number of OTUs was recovered for the 10.0 g sediment sample with all except one primer pair. V4 and COI-A primers attained the highest number of OTUs assigned to taxa (>300 OTUs both), followed by V1-V2, V9, and COI-B. Various taxonomic groups were exclusively or preferentially detected by a target region or primer pair. **Significance:** Our results indicate that no single target region or primer pair capture entirely, or expressly, the phylogenetic diversity of an estuarine meiofaunal community. This would reflect either amplification bias or taxonomic gaps in the reference libraries. Considering the current limitation of tools and libraries available, we propose to (*i*) target at least two loci, using one or multiple primer-pairs for each one, and (*ii*) use a fair amount of sediment sample to capture a representative profile of the existing diversity.

Barcoding piranhas and pacus: species diversity and morphological convergence of reofilic taxa

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Background: Fish account for more than 50% of the 54 000+ species of described vertebrates. Freshwater Neotropical ichthyofauna account

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for 13% of this diversity, with over 5000 described species. Yet, it is estimated that at least 30%–40% of Neotropical ichthyofauna still has to be described. In many of these cases, species descriptions are hindered by the difficulty of diagnosing species using morphological characters, as well as unknown levels of intraspecific morphological variation due to ontogenetic changes and phenotypic plasticity. The family Serrasalmidae comprises 90+ taxa that are widely distributed in all major South American river systems. These species inhabit a diversity of habitats from floodplain forests to rapids. **Results:** We analyzed 926 specimens of serrasalmids belonging to 55 nominal species and 11 additional taxa identified only to genus level, representing all 16 living genera of this family. Mean intraspecific divergence values ranged from 0% to 13%, while mean interspecific divergences ranged from 0% to 11%. Only 56% of the nominal species (37) were monophyletic and could be correctly assigned to a morphospecies based on their DNA barcode. The remaining 29 taxa were paraphyletic. Based on standard DNA barcoding, together with analytical methods (GMYC, bGMYC, mPTP), we inferred the existence of 82 species, 24% more than the number of morphospecies. Many of the genera were paraphyletic, principally those comprising species adapted to reofilic habitat, indicating convergence and local adaptation. **Significance:** Overall, our results indicate that serrasalmid diversity is much higher than the number of morphologically recognized species. A large number of cryptic species, species identified in DNA barcode analyses, were observed in the piranha genus *Serrasalmus* and the pacu genus *Metynnis*. Reofilic genera were not monophyletic, and they showed patterns derived from local fauna, highlighting previously unrecognized within-drainage endemicity and uniqueness of these taxa, which currently are under threat from the implementation of large hydroelectric infrastructure projects.

Next generation wildlife monitoring: a comparison of eDNA and camera trapping in Kruger National Park, South Africa

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Background: Recent technological advances have expanded options for non-invasive monitoring of vertebrates for conservation purposes. Due in large part to decreased cost coupled with increased sensitivity over the past decade, two approaches have gained in popularity: camera trapping and metabarcoding techniques based on sequencing of environmental DNA (eDNA). Both approaches have unique advantages and may provide complementary information when building local species inventories. Camera traps provide a time stamped record of visitations and allow for estimation of group sizes and abundance-weighted species associations. eDNA allows for discrimination among cryptic species that may not be detectable with camera traps. To date, these approaches have yet to be directly compared. **Results:** We present a case study documenting watering hole visitation by mammals in Kruger National Park, South Africa (KNP). We verify the ability of eDNA metabarcoding to detect vertebrate signals in water by comparing to direct observation with camera traps. We also examine the influence of body size and visitation patterns on eDNA detection rates for medium and large mammal, and discuss the benefits and limitations of both approaches in this system. **Significance:** This is the first study to use camera trap documentation to verify species detections based on eDNA metabarcoding of watering holes. We present the advantages and challenges associated with each approach, and discuss future directions for biodiversity monitoring and in African savannah ecosystems, including the extension of barcoding to microorganisms to identify host-associated species, and quantification of the potential for disease transmission among hosts.

The good, the bad, and the ugly: insights from Odonata barcoding

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DNA barcoding is currently an essential tool in a vast array of ecological and conservation studies (e.g., biodiversity monitoring, diet assessments). However, its applicability is still hampered by the lack of comprehensive reference collections. This knowledge gap becomes greater in invertebrates, especially from biodiversity hotspots like the Mediterranean Basin. Surprisingly, while dragonflies and damselflies are one of the best-studied insect groups, no comprehensive barcoding of the European species has been made. These predatory insects are intimately connected to freshwater habitats, as their larval phase is completed in the water, being particularly sensitive to changes in the aquatic environment and constituting important bioindicators of ecosystem health. Within InBIO Barcoding Initiative we barcoded more than 70 species of odonates, focusing mostly on species from the Iberian Peninsula. Genomic DNA was extracted, and the barcoding mitochondrial COI gene fragment (658 bp) was amplified. DNA barcodes were sequenced using either Sanger or high-throughput sequencing (Illumina). Our results exhibited a scenario that illustrates some of the challenges posed by insect identification using DNA barcoding. While many species can be easily identified using the mitochondrial COI gene fragment, this is not true in all cases. Not all species possess a specific DNA barcode that allows the correct assignment of taxonomic names to unidentified specimens. For instance, two groups of coenagrionid species share mtDNA haplotypes. Other species possess multiple copies of COI in the genome, impeding successful Sanger sequencing, which can be overcome using next-generation sequencing. These sequences are also likely to be detected in environmental DNA (eDNA) metabarcoding studies, and should, therefore, be documented and databased for more accurate estimation of taxa diversity and species identification. These data provide important insights into the diversity and taxonomy of odonates and guidelines to achieve a more reliable and useful barcode reference database.

Large-scale barcoding of Portuguese moths: accelerating species inventories while revealing exotic species, sexual dimorphism, and cryptic diversity

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Lepidoptera is a highly diverse order of insects with over 2500 species listed for Portugal. Moths have a wide ecological role as they act as pollinators and are prey of many organisms. Moreover, some species cause significant impacts on agriculture and, therefore, developing a cost-efficient monitoring scheme is fundamental. Molecular identification of taxa using DNA metabarcoding is expected to have high applicability in biodiversity monitoring and ecological research. However, it is dependent upon the existence of comprehensive DNA barcode reference collections. In the Mediterranean region, such comprehensive database is still lacking. In this context, and within the frame of InBIO Barcoding Initiative, we are developing a DNA barcoding database. So far we have collected and analyzed more than 1500 specimens of over 60 families of Lepidoptera, with a total of over 1000 species barcoded. Genomic DNA was extracted and the mitochondrial COI gene fragment (658 bp) was amplified in two overlapping fragments and sequenced using high-throughput techniques (Illumina). Most species could be easily distinguished using the targeted sequence, but some cases of low divergence between species

were detected. Furthermore, DNA barcodes facilitated the correct identification of enigmatic specimens, either of undocumented species in the region (both indigenous and exotic) or linking males and females of sexually dimorphic species. Cryptic diversity was found in several situations, especially when comparing specimens from Iberia and central Europe. The development of InBIO's Lepidoptera reference collection is driven primarily by a research line on the study of diets and the building of food webs. The barcoding of Portuguese Lepidoptera is directly assisting the application of metabarcoding techniques towards a better understanding of food web complexity in Mediterranean ecosystems and the identification of trophic relationships relevant for pest management. We expect InBIO's reference collection to become a fundamental tool for long-term and large-scale biodiversity monitoring in the Iberian Peninsula.

DNA barcoding of wild and edible insects to sustain forests and fight malnutrition in Madagascar

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Insects are everywhere and diverse, yet they are invisible in most conservation efforts. Since terrestrial ecosystems make no sense except in light of insects, this is short-sighted and unfortunate. The time is now for entomologists to end their silence on the loss of insect habitats. After all, how much tropical forest will be left in 50 years? Insects and People of the Southwest Indian Ocean (IPSIO) advocates insect research as central to successful conservation outcomes in the Malagasy region. DNA barcoding underpins IPSIO's principal projects, including the farming of edible insects and the development of environmental assessment tools. By DNA barcoding all species of edible insects used by the 18 ethnic groups in Madagascar, IPSIO aims to enhance food security in an area plagued by malnutrition while encouraging forest conservation. The goal is to identify arthropod species that can be farmed at a commercial scale. IPSIO is also establishing barcode libraries for target insect groups to enable their use as indicators in environmental assessments and conservation mapping. To date, these barcoding efforts are most advanced in ants (3400 BINs for 19 752 sequences), butterflies (278 BINs for 824 sequences), dragonflies (12 BINs for 29 sequences), millipedes (41 BINs for 93 sequences), Sphingidae (39 BINs for 53 sequences), Saturniidae (13 BINs for 95 sequences), and water beetles (169 clusters for 2043 sequences). The IPSIO network demonstrates the role and importance of DNA barcoding of insects in conservation and, at the same time, drives new interest in insect exploration.

Towards a genomic approach to plant authentication and quality control

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Advances in DNA technology, the decrease in costs, and increased availability of equipment have meant that DNA methods are now starting to be considered as viable tests to be included in national pharmacopoeias, along with the traditional morphology and chemical tests. The use of pre-designated DNA barcodes has been widely advocated in the past decade as the golden standard in DNA-based authentication, but it has not been constantly successful at identifying a particular sample at species level, particularly in plants. Furthermore, the approach itself is not always suitable for degraded material as often the template requiring identification in the trade of traditional medicines, for example, is recalcitrant to traditional methods. The advent of high-throughput sequencing (HTS) technologies offers new tools in DNA-based authentication that promise to resolve some of the issues related to the use of more traditional DNA barcoding. It is clear that DNA methods, particularly those using HTS technologies, along with chemical methods, provide approaches that can discriminate among species of plants as well as provide an indication of quality. They can also identify contaminants, harmful substitutes, and adulterants. Here, we discuss the development of a genomic approach combining genome skimming and targeted enrichment using an angiosperm-wide bait set. We also show how the combination of genomics and chemical profiling opens new opportunities for plant authentication and quality control.

Utilizing pollen DNA metabarcoding for reconstructing pollinator networks in forests managed for biofuel production

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Background: Current knowledge of plant–pollinator visitation networks is based primarily on visual sightings of bees coming and going from various flowers in one location and can lack both accuracy and time depth. When pollen from bees is identified to provide information on prior foraging, plant–pollinator networks become more detailed. Further advantages, in efficiency and accuracy, can be achieved when this is coupled with DNA metabarcoding to identify mixed species pollen batches and high-throughput sequencing. In our experiment, we used dual-indexing DNA metabarcoding with the *rbcL* and ITS2 regions in order to gather data and recreate a pollinator network in forests being managed for biofuels production in Florida. **Results:** We were able to successfully construct a quantitative pollinator network using DNA metabarcoding. **Significance:** This work demonstrates that non-quantitative DNA metabarcoding can be used in constructing quantitative pollinator networks. This also demonstrates the increased efficiency and cost-effectiveness of DNA metabarcoding for mixed species pollen batches over traditional barcoding or visual sighting methods.

Unlocking the genetic diversity of free-living *Symbiodinium*

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Background: *Symbiodinium* are endosymbiotic microalgae of reef-building corals. Photosynthesis by these algae fuels the productivity of corals and ultimately the growth of entire reef systems. However, a critical phase of the life history of *Symbiodinium* is existing as "free-living" prior to acquisition by their hosts. Despite the importance of free-living populations, their underlying biodiversity and ecology remains a "black box". We therefore applied environmental DNA (eDNA) metabarcoding to resolve the complex phylogenetic diversity across reef habitats (water, sediment, and surface of macroalgae) and latitudes in eastern Australia where in hospite *Symbiodinium* diversity

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is highly divergent. **Results:** *Symbiodinium* is divided into nine clades (clades A–I) and hundreds of subclades. We observed a highly complex and diverse free-living community of *Symbiodinium*, with seven clades (all except E and I) comprised of >30 subclades in the tropical site. In contrast, only five clades were observed in the temperate site, dominated by clades A and B (reflects in hospite community) and rare occurrence of clades C, E, and F. Overlapping of genetic types between free-living and in hospite populations was found in both sites. Interestingly, "exclusively free-living" *Symbiodinium* were also found, where clade A (the ancestral clade) was abundant in the sediment and clade E was only found in the temperate site. **Significance:** DNA metabarcoding has not been widely applied to understand free-living populations of *Symbiodinium*. Our study provides new knowledge on the inherent diversity of putative species that either exists as exclusively free-living or transiently as a part of the symbiotic life cycle. We have provided the first description of free-living diversity associated with temperate reefs, and importance to resolve baseline where such habitats potentially offer refuge to tropical corals subjected to global warming. Our study underlines the close relationship between free-living and in hospite communities where habitats/regions differentially operate in the life history of alternate genotypes of *Symbiodinium*.

Hidden diversity within a Neotropical freshwater fish genus, *Megaleporinus* (Characiformes: Anostomidae), revealed by DNA barcoding

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Background: Molecular studies have been improving our knowledge of the Neotropical ichthyofauna. DNA barcoding has successfully been used in fish species identification and in detecting cryptic diversity. Here, we assessed all nominal species of the recently described genus *Megaleporinus* (Anostomidae) using a DNA barcode approach with a broad sampling, to generate a reference library, characterize the molecular lineages, and test the hypothesis that some of the nominal species represent species complexes. **Results:** The GMYC analysis identified 18 different molecular operational taxonomic units (MOTUs) within the 10 studied nominal species, indicating cryptic biodiversity and potential candidate species. Only *Megaleporinus brinco*, *Megaleporinus garmani*, and *Megaleporinus elongatus* showed correspondence between nominal species and MOTUs. Within six nominal species a subdivision into two MOTUs was found, while *Megaleporinus obtusidens* was divided in three MOTUs, proving that DNA barcoding is a very useful approach to identify molecular lineages in *Megaleporinus*, even in the case of recent divergence. **Significance:** Our results thus provide molecular findings that can be used along with morphological traits to better define each species, including candidate new species. This is the most complete analysis of DNA barcoding in this recently described genus, and, considering its economic value, a precise species identification is desirable and fundamental for conservation of the biodiversity of this fish.

Barcoding life in the Cape: insights from the phylogeography of a small Cape genus

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Background: The Cape is synonymous with exceptional plant species richness. Hosting the smallest and most diverse floral kingdom, it is no surprise that the evolutionary drivers behind these patterns have long fascinated biologists. It has been widely accepted that, while perhaps not the cause of the observed diversity, the historically stable climatic conditions of the Cape have buffered this region from major extinction and recolonization events. This has allowed for the persistence and evolutionary radiation of ancient lineages along multiple ecological gradients. This has resulted in the assembly of communities with an equally fascinating molecular history, where ancient taxa occur alongside relatively recent products of evolutionary adaptation. However, what do these different evolutionary timescales mean for those attempting to barcode the diversity of the Cape flora? **Results:** Here, I will report on insights gained from investigating the phylogeny and phylogeography of the relatively young Cape genus *Cyclopia* Vent. and highlight some of the issues of applying traditional barcoding approaches to Cape taxa with poorly resolved evolutionary histories. In addition, I will also address the use of High Resolution Melt analyses, a cost-saving technique for the large sample sizes required for phylogeographic studies.

Uncovering bark: the use of DNA barcoding to identify unknown bark species illegally traded at the Faraday traditional medicinal market in Johannesburg

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Background: The illegal trade of bark from indigenous plant species is a growing trend that goes hand in hand with traditional belief systems in South Africa. Certain barks are thought to have medicinal and magical properties and are widely used as an alternative to Western medicines. The growing demand for traditional medicines has inevitably led to unsustainable and careless harvesting practices by traditional healers and collectors who are often unaware of environmental laws and regulations. It has also led to some species being adulterated or substituted with others, which can pose serious health risks. Furthermore, the barks are often processed in such a way that identification by conventional methods is impossible. Here, we employed DNA barcoding to authenticate bark and bark-derived products sold at the Faraday traditional medicinal market in South Africa. **Results:** The current reference data set comprises over 1400 woody plant species of southern Africa, which represent 66% of the ~2200 species (115 families and 541 genera) in the region. This data set was used to identify and assign species names to bark samples and their derivatives obtained from the market. **Significance:** This study highlights threatened species found at the market. Furthermore, the study also aimed to implement viable, educational solutions that will focus on environmental laws and the importance of biodiversity in the country.

Can phenotypic differences predict genetic clade membership in the ultramarine grosbeak (*Cyanocompsa brissonii*)?

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Background: The ultramarine grosbeak (*Cyanocompsa brissonii*) is a songbird of the family Cardinalidae endemic to South America. It is divided into five subspecies, based on morphology and plumage coloration. A regional-scale study of DNA barcodes showed that there are two clades of *C. brissonii* in Argentina, with more than 2% genetic divergence and no apparent geographical barriers that could impede the gene flow between them. Here, we analyzed variation in COI as well as in other mitochondrial and nuclear markers and assessed if phenotypic variation (morphology, plumage coloration, and vocalizations) correlated with genetic variation. **Results:** We found two reciprocally monophyletic mitochondrial clades with high to maximum support within *C. brissonii*. Congruent with previous findings, Clade 1 grouped individuals from northwestern Argentina, western Paraguay, and Bolivia, while Clade 2 included samples from northeastern Argentina, Brazil, and Colombia. Mean divergence between clades was 2.43%, while average distance within them was 0.15% (Clade 1) and 0.51% (Clade 2). No distinguishable clades were found using nuclear markers. In Corrientes province (Argentina) both clades meet, and the

area where individuals of both clades can be found extends at least from the Paraná River to the Mburucuya National Park (~50 km). Individuals from this area could not always be assigned correctly to their genetic clade based on their phenotype, contrary to what happened with individuals from other regions. **Significance:** It has recently been estimated that the actual number of bird species might be twice that currently recognized. The correct delimitation and quantification of species is fundamental for evolutionary studies and conservation efforts. This study shows that DNA barcodes can play a fundamental role in detecting cryptic diversity and assessing species boundaries. It is worth mentioning that this is the first time individuals from the isolated population of Colombia are included in molecular analyses.

DNA barcodes reveals that the monogonont rotifer *Brachionus quadridentatus* is a species complex

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Background: Because of their size, phenotypic variation, and occasional morphological stasis, monogonont rotifers are difficult to identify to species level. In addition, previous DNA barcoding studies on rotifer species has revealed the presence of cryptic species. Therefore, evidence of diversity within rotifers still needs to be investigated. *Brachionus quadridentatus* is a cyclical parthenogenic rotifer, which inhabits fresh and brackish water worldwide. This species exhibits extensive intraspecific morphological variation associated with spine development, and, in consequence, different forms and varieties have previously been described in this taxon. However, the taxonomic status of these variants needs to be comprehensively investigated. **Results:** Barcodes were obtained from 176 individuals of *B. quadridentatus* collected from Mexico. Our phylogenetic analyses discriminated seven genetically distinct lineages (BqI–BqVII), which are highly congruent with seven morphotypes identified in this study. Genetic divergences within the seven genetic lineages ranged from 0% to 3.1%, while divergences among the seven lineages ranged from 12% to 18%. Our results are evidence of the underestimated diversity in *B. quadridentatus*, and indicate that this species represents a species complex. However, more sampling is needed in areas between distant locations and outside of Mexico in order to reveal the extent of genetic differentiation and diversity within this taxon. **Significance:** This is the first study conducted to explore the diversity within *B. quadridentatus*, and it highlights the need for further investigation in order to assess species boundaries and to recognize cryptic lineages in *B. quadridentatus* as valid species.

Needs and challenges for a DNA barcoding study of the Libyan flora

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Previous case studies, using native *Malva* and Mediterranean *Arbutus* (Ericaceae), have shown the need for a comprehensive review of the Libyan flora. Here, we discuss the state of the known flora, current work to update the listing of species, and highlight the large gaps in DNA data for Libya. The national need for a DNA barcoding approach was recognised through a lecture at Benghazi University and in an interview on Libyan national television in early 2017. There remains the obstacles of infrastructure and finance for such an ambitious project; however, the potential advantages of an updated flora backed by a DNA barcoding approach may revitalise floristic botany nationally, offer new insights into native biodiversity, and allow Libya to better fulfil Convention on Biological Diversity (CBD) targets. Approaches to developing a network of researchers to study the floras at the national level and to streamline the processes of description and discovery are discussed. Priority taxa for initial study are suggested.

DNA barcoding the flora of Qinling Mt. in China

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Background: The Qinling Mountain Range covers an area of ~76 500 km^2 and ranges from 32°5′N to 34°45′N and from 104°30′E to 115°52′E, with the highest peak about 3767 m above sea level. This mountain is a natural boundary between subtropical and warm temperate region in China. Its flora consists of 3839 species (164 families, 1067 genera), among them, 69.4% genera (740) with 1 or 2 species; in contrast, 75.9% species (2912) are from big genera with more than 3 species. **Results:** In this study, we collected 1600 specimens and generated DNA barcodes (*rbcL*, *matK*, ITS) for 1137 plant species from 592 genera of 137 families. Among these samples, there are 603 woody species and 534 herbs. Also, 359 species from 221 genera are endemic to China. **Significance:** This DNA barcode library could provide valuable data for many related studies on ecology, conservation biology, and biogeography studies.

Global inequities and sharing genetic resources for non-commercial research: a case study of the DNA barcode commons

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Background: Life sciences research that uses genetic resources is increasingly collaborative and global, yet collective action remains a significant barrier to the creation and management of shared research resources. These resources include sequence data and associated metadata, and biological samples, and can be understood as a type of knowledge commons. Collective action by stakeholders to create and use knowledge commons for research has potential benefits for all involved, including minimizing costs and sharing risks, but there are gaps in our understanding of how institutional arrangements may promote such collective action in the context of global genetic resources. We address this research gap by examining the attributes of an exemplar global knowledge commons: The DNA barcode commons. DNA barcodes are short, standardized gene regions that can be used to inexpensively identify unknown specimens, and proponents have led international efforts to make DNA barcodes a standard species identification tool. Our research examined if and how attributes of the DNA barcode commons, including governance of DNA barcode resources and management of infrastructure, facilitate global participation in DNA barcoding efforts. Our data sources included key informant interviews, organizational documents from iBOL and CBOL, a dataset of 3557 articles published by the DNA barcoding community, and DNA barcode record submissions for disease-carrying mosquito genera and medicinal plants. **Results:** Our research suggested that the goal of creating a globally inclusive DNA barcode commons has not yet been fully achieved, and that the risks and benefits of participating in the commons are not equitably shared across heterogeneous global participants. **Significance:** DNA barcode organizations can mitigate the challenges caused by its global membership through ensuring its governance is representative and considers restrictions on use that may enhance participation in the commons.

Overview on the activities in the German Barcode of Life Project Phase II

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Since November 2011, the German Ministry of Education and Science (BMBF) is funding a consortium of natural history museums and research

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institutions to set up the German Barcode of Life (GBOL) initiative. The main goal was to establish a network of professionals and non-professionals to begin with the construction of a DNA barcode reference library for the fauna, flora, and fungi of Germany. Most project goals of the first phase (2011–2015) have been achieved: a national web portal for DNA barcodes and specimen data was developed and is continuously improved (www.bolgermany.de); over 250 independent scientists provide their taxonomic expertise and over 50 institution-based taxonomists contribute to GBOL. Especially, the engagement of external experts contributed significantly to the project's success: of the 48 000 animal and 10 000 plant species (excluding algae and fungi) present in Germany, over 23 000 different species plus a few selected rust fungi (Pucciniales) have been processed, and DNA barcodes for them were generated. In total, 295 000 specimens were submitted to GBOL institutes, and after choosing (usually) up to 10 individuals per species from throughout their distribution range in Germany, over 145 000 of them delivered a DNA barcode. The second phase of the initiative (2016–2018) is now focusing more on applications of DNA barcoding with seven dedicated PhD students working on specific aspects from metabarcoding for water quality assessments to developing a diagnostic microarray chip for the detection of phytopathogenic fungi. As a prerequisite for the successful implementation of the new techniques, a core team and network of taxonomists are further expanding the reference library with DNA barcodes for another 13 800 species. With this target the database will be filled with about half of the known metazoan species of German animals and plants and be operable to identify the vast majority of organisms in terrestrial and aquatic environmental samples.

Testing the Global Malaise Trap Program: how well does the current barcode reference library identify flying insects in Germany?

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Background: Biodiversity patterns are inherently complex and difficult to comprehensively assess. Yet, deciphering shifts in species composition through time and space are crucial for successful management of ecosystem services, as well as for predicting change. To better understand species diversity patterns, Germany participated in the Global Malaise Trap Program, a worldwide collection program for arthropods using this sampling method followed by DNA barcode analysis. Traps were deployed at two localities: Nationalpark Bayerischer Wald in Bavaria, the largest terrestrial Natura 2000 area in Germany, and the nature conservation area Landskrone, an EU habitats directive site in the Rhine Valley. Arthropods were collected from May to September to track shifts in the taxonomic composition and temporal succession at these locations. **Results:** In total, 37 274 specimens were sorted and DNA barcoded, resulting in 5301 different genetic clusters (BINs, Barcode Index Numbers) with just 7.6% of their BINs shared. Accumulation curves for the BIN count versus the number of specimens analyzed suggest that about 63% of the potential diversity at these sites was recovered with this single season of sampling. Diversity at both sites rose from May (496 and 565 BINs) to July (1236 and 1522 BINs) before decreasing in September (572 and 504 BINs). Unambiguous species names were assigned to 35% of the BINs (1868), which represented 12 640 specimens. Another 7% of the BINs (386) with 1988 specimens were assigned to genus, while 26% (1390) with 12 092 specimens were only placed to a family. **Significance:** These results illustrate how a comprehensive reference library can identify unknown specimens, but also reveal how this potential is constrained by gaps in the quantity and quality of records in the Barcode of Life Data System (BOLD), especially for Hymenoptera and Diptera. As voucher specimens are available for morphological study, we invite taxonomic experts to assist in the identification of unnamed BINs.

Authenticating fish and seafood products for sale on the Belgian market

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Background: Due to the international importance of fish and seafood trade, there is a large potential for (un)intentional misidentification and (or) deliberate fraud through species substitution. Several studies worldwide indicate that this is common practice, especially for processed products that lack characterizing morphological features (e.g., fillets). More stringent regulation on foodstuff labelling is supposed to enhance traceability, and protect consumers and the seafood industry from (un)intentional mislabeling. For Belgium, which has a higher per capita fish consumption than the EU average and a seafood import rate of 54% (42% of the total import comes from outside the EU), a study on samples collected from restaurants exposed a 32% incidence of fish mislabeling. In the present study, we sampled fish and seafood at various supermarkets and fishmongers to evaluate the frequency of seafood mislabeling on the Belgian retail market including a broad range of taxa and processing methods (e.g., fresh, frozen, smoked, pickled, cooked, fried). **Results:** Due to the large range of taxa being analyzed, several technical aspects concerning marker choice, primer selection, protocol optimization, and interference as a result of food processing are being encountered, analyzed, and improved. Preliminary identification results uncovered mislabeling of several samples; however, at this stage, there does not yet appear to be a pattern towards specific taxa or treatments. **Significance:** Although past seafood fraud studies investigated different taxa, processing methods, and purchase locations, they all seem to indicate that the scale, as well as the product most prone to mislabeling, differs by country. The present exploratory survey, including a broad taxonomic range of seafood species, therefore aims to identify those fish and seafood products (species and processing) most subjective to mislabeling in Belgium, while also further optimizing the identifications techniques.

Aliens in Europe

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Background: An increasing number of species are being introduced into Europe, either by accident or deliberately. Some are able to establish viable populations and may outcompete other species or disrupt ecosystem functioning: these species are called invasive alien species (IAS). In order to (*i*) protect native biodiversity and ecosystem services, and (*ii*) mitigate potential impacts on human health and socio-economical activities, the European Commission issued *Regulation 1143/2014*, reporting on 37 IAS. The Regulation foresees three types of interventions: (*i*) prevention, (*ii*) early detection and rapid eradication, and (*iii*) management of established populations. Aside from compiling this list and gathering information on presence, distribution, ecology, impacts, and management, accurate methods for rapid identification are required when suspicious biological material is being encountered. In cases where a morphological identification is problematic (e.g., cryptic species, trace material), DNA-based identifications may represent an alternative method. The purpose of the present work is, therefore, to investigate and evaluate the available molecular identification techniques for each IAS in silico. **Results:** We investigated the usefulness and accurateness of the Barcode of Life Data System (BOLD) (COI for animals; *rbcL*,

ITS2, and *matK* for plants) and encountered some limits when using BOLD barcodes as the only tool for species identification. Knowledge gaps regarding (*i*) the sequence coverage of the IAS and their sister species, and (*ii*) the metadata on the vouchers (e.g., subspecies, locality) have been detected, which can hamper reliable identification. We therefore investigated the feasibility to complement the BOLD database, as well as the usefulness of complementary markers and methods (e.g., RFLP) in providing reliable and rapid identifications. **Significance:** The present project aims to provide an up-to-date status on the molecular tools and methods available for rapid and accurate identification of IAS, as well as to optimize and complement them whenever necessary.

NaturaeData: a morphological and DNA barcoding regional library

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Background: Although Brazil is a hotspot for biodiversity, information made available to identify animal species is still lacking and (or) unavailable for the general public (particularly due to language constraints). Additionally, the data available to BLAST sequences mainly come from northern hemisphere species deposited in Genbank and the Barcode of Life Data System (BOLD), despite the massive DNA sequencing initiatives executed in the last 10 years by a number of Brazilian research groups. Morphological characters used to identify such species have not yet been explicitly integrated with barcoding in the existing databases. **Results:** We have developed an online public local library named NaturaeData (www.naturaedata.com.br), starting with phytophagous true bugs (Pentatomidae), microlepidoptera (Cecidosidae and Gracillariidae), and small rodents (Cricetidae and Didelphidae). They include high-resolution images of adult specimens and their genitalia, as well as the morphological characters used to diagnose them. **Significance:** This is the first regional database of animal species from southern Brazil. We provide an online library with morphological diagnostic characters and DNA barcoding sequences that allow for the identification of species by researchers and general audiences, in Portuguese. Thus, we propose an opposite initiative compared to the massive and fast sequencing output of species currently found in diversity inventories. Simply put, we set forth a slow and steady online process, which includes morphological descriptors that aid in animal species identification by taxonomists.

Colombia BIO: discovering biodiversity in post-conflict territories in Colombia

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After more than 50 years of armed conflict in Colombia, the signing of a peace agreement has opened territories to the exploration of biodiversity. These newly open areas harbor an amazing biodiversity but are under major transformation pressure. With a team of 30 researchers, three major areas of high-predicted diversity, previously unexplored, were sampled during 10 days with particular focus on the groups of birds, amphibians, reptiles, insects, fungi, fishes, mammals, and plants. We barcoded all sampled specimens, generating 5000 sequences that double the current information available for Colombia in the Barcode of Life Data System (BOLD). The first expedition took place in a karst system, while the others were in mountain forest and savanna. By combining DNA barcodes and taxonomic expertise we have confirmed the discovery of nine new species from the karst system including a blind fish, a snake, and two amphibians. Major discoveries are in progress for the other two places. While increasing knowledge for one of the most biodiverse countries in the world, this study helps to provide a new identity for the human communities that were severely impacted by war and who see biodiversity as a new alternative for living.

Revealing the diversity of deadly venomous caterpillars from the genus *Lonomia* (Saturniidae: Hemileucinae) and its epidemiological implications

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Background: Several families of Lepidoptera (moths and butterflies) are known to be of public health concern. Within family Saturniidae, caterpillars of the Neotropical genus *Lonomia* are responsible for fatal envenomation of humans in South America. This is caused by the procoagulant action of their venom after skin contact with the caterpillars' spines. Until now, only two species have been reported as causing incidents of medical importance: *Lonomia achelous* and *Lonomia obliqua*. However, species identification has been largely unquestioned despite knowledge of venom diversity, and growing evidence that the current taxonomy misrepresents species diversity in the genus. Of the 46 currently recognized species, 31 have been described post-2011 and all need their taxonomic status, distribution, and larval toxinology clarified. **Results:** Our study addresses species diversity in genus *Lonomia* using an integrative approach combining DNA barcodes and morphology for ~1200 specimens from both natural history collections and specimens newly collected in 2015–2016 in Colombia and Brazil. Following discovery of new species and cases of putative synonymy, the taxonomy is revised. Potential species distributions derived from spatial distribution models are presented. We also report interspecific variation of larval toxinology, which has revealed that not all species of *Lonomia* are of equal public health concern. **Significance:** Our results shed new light on the diversity of genus *Lonomia* and more specifically clarify the distributions of those species known to cause the most serious envenomation incidents. Despite recent advances in the assessment of species diversity in this genus, our study revealed at least three undescribed species, emphasizing the need for further research into these medically important moths.

Don't judge book by its cover: the case of freshwater gammarids of the Periadriatic region

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Background: Modern molecular studies reveal the presence of very high levels of cryptic diversity and local endemism in many freshwater taxa. The current extinction rate of freshwater species is estimated to be at least 5× higher than that of terrestrial species, mostly due to heavy anthropogenic degradation of freshwater ecosystems. This leads to the situation where the rate of extinctions in freshwater habitats will exceed the speed at which new species are identified and described. The Mediterranean, including the Periadriatic Region, is one of the most important biodiversity hotspots on the global scale. Freshwater gammarids are widely used as model organisms in ecotoxicological and biomonitoring studies. However, the majority of the studies on gammarid crustaceans of the region have focused mainly on marine species and neglected the freshwater fauna. **Results:** Sev-

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eral sampling campaigns in the years 2005–2016 yielded a very large collection of gammarids, encompassing more than 40 000 individuals gathered from over 500 inland and insular locations in the Periadriatic region. Molecular analyses revealed an extremely high level of cryptic diversity, greatly exceeding the number of morphospecies already described from the region, i.e., 40 molecular species within the *Gammarus balcanicus* group, at least 12 species within *Gammarus roeselii*, around 10 cryptic species within *Gammarus italicus*, as well as 10 molecular species of *Gammarus* from the Peloponnese and high cryptic diversity including a few dozen species within *Echinogammarus* in the Apennine Peninsula. **Significance:** The collected data greatly aids in revealing the biogeographical affiliations and the diversification time frames of the local gammarids and will eventually help to update and revise the taxonomy within the family Gammaridae. Knowledge about the real level of biodiversity will greatly aid planning reasonable and effective strategies preventing or minimalizing the rapid loss of freshwater biodiversity, due to the extensive anthropogenic degradation of the local freshwater ecosystems.

DNA barcodes as a tool for the identification and control of illegal wildlife trade: a case study of Colombian mammals

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Background: Illegal wildlife trafficking of endangered species is one of the prominent causes of global biodiversity decline. This illicit practice may involve intact individuals; however, processed, transformed, and parts of the invidual are more commonly used. The latter practice poses challenges for correct species identification by traditional morphological methods and complicates commerce regulation. DNA barcoding and DNA extraction protocols for a wide variety of animal tissues are a useful tool not only for rapid and positive species identification, but also for regulating wildlife illegal trafficking. **Results:** This study presents the results of the first DNA barcode reference library for Colombian mammals threatened by illegal trafficking. Moreover, it evaluates the applicability of DNA barcodes as a tool for controlling such practices. We provide the first barcoding sequences (cytochrome *c* oxidase subunit I (COI)) for 36 mammal species. During the study, various Phenol-Chloroform DNA extraction protocols were evaluated and standardized by using diverse animal tissues (e.g., soft tissues, cartilage, claws, horns, thorns, bones, teeth, and hair) obtained from museum specimens. Finally, information acquired here was used in a real illegal trafficking case for which real-time DNA sequencing using Nanopore technology was successfuly used in identifying the trafficked animal. **Significance:** This investigation elucidates (*i*) a DNA extraction protocol evaluated and standardized for completely variable animal tissue samples (i.e., transformed, degraded, and ancestral DNA), and (*ii*) a DNA barcode reference library for mammals involved in illegal trafficking. Moreover, it generates a tool for illegal trafficking authorities to control this practice worldwide.

Root endophyte communities differ between sodic and non-sodic soils in a catena ecosystem of the Kruger National Park, South Africa

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Background: Granite catena systems form part of super sites in Kruger National Park, South Africa, and they are the focus of intensive research promoting conservational practices. In such a system different ecotypes occur, each typified by unique soil and hydrological properties, and distinct differences in faunal and floral communities. It is, however, unknown how the different ecotypes influence internal fungal communities of the plants present in each ecotype. This study determined if the fungal endophytic community composition from roots of particular herbs occurring in adjacent sodic and non-sodic soils of one such supersite were similar or different as reflected by the differences in soil conditions. Mini-barcodes generated by next-generation sequencing (NGS) technologies allow for the rapid classification of microbial communities. An Illumina platform was used to sequence the fungal ITS2 region of the ribosomal operon from 20 plants collected from each site, and for two co-occurring herbaceous plants. **Results:** Distinct differences in the fungal root communities of the plants were observed between the soil sites. For example, a number of genera found in roots from one soil site were not identified at the other soil site. Results suggest that soil types influence the fungal communities, such as those occurring in a plant able to grow under the different soil conditions. **Significance:** Implications for conservation are that even if plant hosts are not sensitive to different soils, soils may have an influence on the fungal communities inhabiting below-ground parts of these plants.

Using DNA barcodes to predict biodiversity priorities of macrofungi in South Africa

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Macrofungi are fungi large enough to be seen with the naked eye. South Africa has a rich fungal biodiversity, which is unfortunately poorly studied due to a lack of human capacity and expertise. Although some species have been identified, the majority are new or dubiously named and can thus not be used in datasets. Therefore, fungi are rarely included in conservation and biodiversity initiatives. It also frustrates a large and growing group of citizen scientists eager to identify fungi they encounter. The taxonomic dilemma is too great to wait for species to be adequately named and described before fungi can be rightfully included in national and international initiatives. The tools provided by barcoding are ideal to catalogue and characterize our fungi. Moreover, it can be predictively used to determine locations, indicate hotspots, or highlight gaps in our knowledge. The latter is useful to plan future surveys and strategies for more complete assessments. Using two locations, namely Bloemfontein and Pretoria, DNA barcodes were generated from fruiting bodies. These data were supplemented with environmental samples (humus, soil, dead plant material) where mini-barcodes for the currently accepted barcode regions were generated using Illumina sequencing. The complimentary full and mini-barcodes were used in phylogenetic diversity analyses to ascertain the diversity for these two areas and to test if especially the environmental method can be used to accurately detect macrofungi from the environment. In both areas a high level of diversity was detected. This is especially useful in Bloemfontein, where fruiting body development is restricted due to relatively dry conditions. Operational taxonomic units represented by the mini-barcodes could also be used to map the occurrences of macrofungal species. The approaches developed in this study can thus be useful in the future to generate much-needed biodiversity data for South African macrofungal species.

Community structure of epiphytic and endophytic fungi of mangroves using high-throughput sequencing of ITS2 barcode

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Background: Epiphytic and endophytic fungi are widely distributed in ecosystems and play an important role in ecosystem functioning. However, the difference in epiphytic and endophytic fungal commu-

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nities of mangroves has been less documented. **Results:** The epiphytic and endophytic fungal communities associated with the leaves of six mangrove species (*Aegiceras corniculatum*, *Avicennia marina*, *Bruguiera gymnorrhiza*, *Excoecaria agallocha*, *Kandelia candel*, and *Rhizophora stylosa*) in south China were examined using Illumina Miseq sequencing of ITS2 barcode. A total of 650 fungal operational taxonomic units (OTUs) were obtained, including 434 Ascomycota, 157 Basidiomycota, and 59 unidentified fungi. Of the 650 fungal OTUs, 390 were epiphytic fungi, 532 were endophytic fungi, and 272 were shared between them. At class level, Dothideomycetes, Tremellomycetes, and Microbotryomycetes were dominant in both epiphytic and endophytic fungi. The OTU richness of endophytic fungi was significantly higher in *A. corniculatum* than in *B. gymnorrhiza*, *K. candel*, and *R. stylosa*, but this parameter of epiphytic fungi was not significantly different among the plant species. The OTU richness of endophytic fungi was significantly higher than that of epiphytic fungi in *A. corniculatum*, *A. marina* and *E. agallocha*. The community composition of epiphytic and endophytic fungi was significantly different. Furthermore, the community composition of endophytic fungi was significantly different among the plant species, yet there was no significant difference in epiphytic fungi. The host preference of endophytic fungi was much higher than that of epiphytic fungi. **Significance:** This study, for the first time, shows much higher diversity of epiphytic and endophytic fungal communities of mangroves using high-throughput sequencing of ITS2 barcode. Plant identity had significant effect on the community structure of the endophytic fungi, but not on the epiphytic fungi in the natural mangrove ecosystem.

Assessing diversity of brackish water and marine organisms of Sundarbans mangrove forest of Bangladesh through DNA barcoding

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Background: DNA barcoding is an advanced tool to identify unique, cryptic, and new species from aquatic ecosystems and reveals more undisclosed biodiversity than previously estimated. The largest mangrove ecosystem of the world, the Sundarbans, is a transition zone between freshwater of the Ganges and saline water of the Bay of Bengal, resulting in assemblances of an unrivalled aquatic biodiversity. The forest has been declared as world heritage site by UNESCO. The present study aims to evaluate the applicability of the mitochondrial COI gene for accurate identification and building a reference library of DNA barcodes of the brackish water and marine fauna of Sundarbans, Bangladesh. For this purpose, 150 species of fish, 20 species of crabs, and 33 species of molluscs were collected from the aquatic environment of Sundarbans from November 2015 until now. The collected samples were first identified by examining morphometric characteristics and then assessed by DNA barcoding. **Results:** Until now, 80 fish and crustacean species have been successfully barcoded. The study provided first record of two fish species, viz. *Upeneus vittatus* Forsskal, 1775 and *Lagocephalus guentheri* Miranda Ribeiro, 1915 and one crab species, *Charybdis affinis* Dana, 1852 in Bangladesh. Additionally, the study resolved the misidentification of mud crab species of Sundarbans. In most of the literature of Bangladesh, the mud crab species was mentioned as *Scylla serrata*. However, DNA barcoding revealed that the mud crab in Sundarbans is *S. olivacea*, Herbst, 1796 not *S. serrata*. **Significance:** This study represents an important step of building a reference library of DNA barcodes of marine and brackish water faunal species in Bangladesh. The study particularly will make an updated and revised inventory of fish and other aquatic invertebrates of Sundarbans in Bangladesh.

Scaling up DNA metabarcoding for large-scale spatiotemporal analysis of biodiversity

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Background: High-throughput sequencing (HTS) has generated an unprecedented opportunity for gathering sequence information for a wide range of biological investigations. DNA metabarcoding utilizes HTS platforms to gather DNA barcode sequences from bulk environmental samples such as water, soil, or sediments. Although DNA metabarcoding has gained much momentum as a rapid biodiversity assessment approach, its applicability in large-scale spatiotemporal analysis of biodiversity demands further optimizations. These optimizations must consider the scope of analysis (e.g., targeted taxa vs. general biota) and issues such as selection of marker genes, recovery of sequences, and data analysis. **Results:** We used exemplar cases from water, benthos, and soil to examine various experimental parameters including template DNA, PCR, sequencing, and data analysis. We used comparative analysis against known biodiversity, as well as statistical analysis, to derive an optimal path for generating biodiversity information for targeted and general biota. **Significance:** Our analyses show significant variation in results obtained using different experimental settings. This study, which is built from several years of active development of proof-of-concept and pilot projects, provides a framework for adopting DNA metabrcoding in various large-scale investigations including applications in national/regional biomonitoring programmes.

Using DNA barcodes to study a taxonomically difficult group of ants (Formicidae: Ponerinae) in Argentina

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Background: The ant subfamily Ponerinae consists of primarily predatory species that often exhibit "ancestral" characters including small colonies, worker fertility, and solitary foraging. In addition to diversity in their ecology and reproductive behavior, this subfamily has also been a challenge for taxonomists in terms of determining relationships among genera, placing species in appropriate genera, and estimating diversity in genera with many cryptic species (e.g., *Hypoponera*). In this study, we examined the systematics and distribution of ponerine ants in Argentina. We used DNA barcodes to test hypotheses regarding the delineation of species currently recognized only by morphology, to assess genetic linages among different biogeographic regions, and to link reproductive castes with worker castes. We collected samples from 10 different regions in Argentina, including from Malaise traps from Formosa and Misiones provinces that were active over two years. Additionally, we reviewed material from five museum collections. **Results:** We obtained 340 COI sequences from 434 individuals belonging to more than 40 species from over 10 genera, including difficult to collect genera like *Thaumatomyrmex* and *Platythyrea*. Among identified workers, the mean intraspecific sequence divergence was 1.7%, six times lower than the mean distance to the nearest neighbour (11%). We found more Barcode Index Numbers (BINs) (63) than the identified species (41), with only one case of BIN sharing by morphological species. Eight species showed deep intraspecific divergence (>6%), and half of the cases were within the genus *Hypoponera*. **Significance:** Our results suggest that barcodes are useful for identifying species boundaries and cryptic diversity of ants from a group that has historically been taxonomically challenging. We were also able to associate reproductive castes with their corresponding workers. We discuss how the diversity of reproductive strategies in ponerines might influence genetic structure, highlighting the need to take into account species natural history when interpreting COI results in this group.

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DNA barcoding and ecological survey of the ants of Iguazu National Park: looking at the tip of the iceberg in a biodiversity hotspot

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Background: Understanding patterns of species diversity can only be achieved by long-term research and the integration of taxonomical, ecological, and behavioral data. Here, we studied the diversity and ecology of ants of Iguazu National Park (INP), Argentina, using six sampling techniques. DNA barcodes were used to uncover cryptic diversity, test species/morphospecies delimitation accuracy, and link unidentified male and queen specimens with their worker caste. **Results:** INP houses 195 described ant species and an additional 49 morphospecies. Leaf litter sampling and pitfall traps were the most efficient sampling methods, while surface baiting revealed the prevalence of large predatory species at different times of the day. Comparing baiting to other sampling methods provided information on species co-existence and the presence of possible dominance hierarchies among ant species. We obtained the DNA barcodes of 312 specimens from 124 species (51% of the ants of INP). Our analyses evidenced a clear barcode gap in all species but two, with an average distance to the nearest neighbour of 15.75%, almost eight times larger than the mean distance to the furthest conspecific (2.07%). Eighty-three percent of the sequence clusters obtained with different clustering algorithms (ABGD, RESL, TCS) matched the reference species or morphospecies, while 10% highlighted possible cryptic diversity. In terms of efficacy, this barcode library allowed a correct identification in more than 94% of the species/morphospecies, and to assign a species name to 69% of the unidentified males and queens. **Significance:** This study evidences that DNA barcodes are a valuable tool for identifying the ants of the Atlantic Forest, a global diversity hotspot. Furthermore, our project provides a framework for understanding the ecology and the taxonomic diversity of the ants of this region, including the identification of currently undescribed reproductive castes and the discovery of possible cryptic species.

Detecting adulteration of ground meats using DNA barcoding and ddPCR

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Background: The detection of food fraud requires the selection of appropriate countermeasures. DNA barcoding is a useful tool for identifying ingredient mislabeling and is now routinely used for seafood authentication. However, its application to other commodities has received less attention, in part because barcoding may not be the most suitable method for dealing with mixtures. Here, we test the utility of barcoding to identify mislabeling of sausage samples declared to be composed of a single species of origin, looking for evidence of potential admixture as irregularities in the sequence electropherogram trace files. We further apply droplet digital PCR (ddPCR) to detect admixture, using a panel of markers for beef, chicken, pork, and turkey. We also screened samples for horse meat using a qPCR assay. **Results:** Barcoding confirmed the presence of the species declared on the product label in most cases, although the method also detected some instances of mislabeling where meat species were wholly substituted. Very few examples of trace file irregularities signalling mixtures were revealed. The application of ddPCR and qPCR methods corroborated the barcode results, but also revealed substantial adulteration/contamination in many of the samples that appeared to be correctly labelled using the barcoding methodology alone, suggesting much higher levels of mislabeling. **Significance:** DNA barcoding alone will often fail to detect admixture. Species-specific screens provide greater resolution of mislabeling and offer the potential to deliver relative quantitation of admixture. However, appropriate reference standards are needed to support quantitation. Ultimately, the patterns of admixture revealed in this study have implications for food safety and consumer lifestyle choice, in addition to economic consequences.

Applications of an extensive DNA barcode reference library: NGS-based analyses of mixed and bulk samples

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Background: Here, we present applications and workflows of DNA metabarcoding utilizing next-generation sequencing (NGS) for identification of animal species present in environmental samples. All projects took place within the framework of the German Barcode of Life (GBOL) initiative at the Bavarian State Collection of Zoology (SNSB-ZSM). **Results:** We use NGS techniques for biodiversity monitoring, with the goal of providing an early warning system of invasive and pest arthropod species recovered from traps in the Bavarian Forest National Park. In our food security application, we investigate species compositions of processed food for human consumption, in order to test the potential of metabarcoding for the validation of ingredients and contaminants. Furthermore, we have applied this technique to provide forensic entomologists with a reference library containing forensically relevant arthropod species collected from multiple sites of decomposing organisms, with the goal of relieving the workload and obtaining accurate and rapid results. In an experiment with dead pigs we examined the arthropod community composition in proximity (including soil) to the corpses and how it changed over time. To date, the ZSM has contributed ~19 000 animal species to the German DNA barcode reference library maintained on the Barcode of Life Data System (BOLD), covering a majority of the animal species commonly used in environmental assessments. We have developed a pipeline to process sequence data generated by NGS. It includes quality filtering, paired-end merging, and clustering of similar sequences into operational taxonomic units (OTUs) for comparison by BLAST against a reference database. **Significance:** The extensive Barcode Index Number (BIN)-based reference library enables metabarcoding to identify species in cases where visual identification would be either too time-consuming (e.g., within bulk samples) or impossible because the organisms are present as immature life stages, belong to cryptic species, or are present only in fragments or trace amounts.

A comparative study on the use of traditional DNA barcoding and next-generation sequencing for determining the trophic interactions of herbivorous insects

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Background: Measuring insect–herbivore trophic interactions is challenging, but the emerging field of ecological genomics is helping to overcome previous limitations with its increasing technological advances. Here, the efficiency of traditional single-species barcoding using Sanger sequencing is compared with novel next-generation sequencing (NGS) using an Illumina MiSeq. These two sequencing methods are trialled on the same selection of orthopterans to analyse their

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gut contents when host–plant species and diet breadth are predominantly unknown. Four standard plant DNA barcode markers were tested (*rbcL*, *matK*, *psbA-trnH*, and ITS), and a hybrid method was developed for NGS to allow for multiple marker PCR products to be sequenced simultaneously. **Results:** By sequencing more than one marker in the Illumina MiSeq cell, we increased the DNA fragment diversity which helped to reduce issues caused by low diversity that can cause lower quality reads and yield output. Overall, we found that the NGS method gave much more exhaustive results than the Sanger method in terms of number of sequences obtained. However, the Sanger sequences were longer in length and therefore could be matched more confidently to a plant species or genus level. One major drawback with Sanger sequencing for the use of diet analysis is that it often fails to deliver a readable sequence from a sample when there is DNA from multiple species within the gut-content, a limitation that is overcome by the NGS method. **Significance:** We suggest that only for known specialist species, the Sanger method is a more appropriate approach for measuring trophic interactions. Although both methods have valuable uses across many fields, NGS has a more extensive potential for developing a wider understanding of trophic interactions in ecological systems.

Are we underestimating the number of plant species in the tropics? New insights from population genetics approaches applied on African forest trees

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Background: Tropical forests are renowned for hosting a remarkable diversity, but the estimation of their species richness depends on the species concept used for their delimitation. Hence, species richness can be underestimated by the occurrence of cryptic species or overestimated by taxonomical oversplitting. Phylogenetic approaches are increasingly used to help decipher species delimitation. However, reciprocal monophyly at gene trees requires that the number of generations since speciation largely exceeds the effective population sizes of the sister species. Alternatively, population genetics methods allow to assess reproductive isolation, which is at the basis of the biological species concept. Using the latter species concept, we relied on large-scale genotyping using nuclear microsatellite markers to evaluate species delimitation in several African tree taxa. To this end, we considered that distinct species can be recognized when well differentiated genetic clusters occur in sympatry. **Results:** (*i*) We found no case of oversplitting: even when phenotypically very similar, species distinguished by taxonomists formed distinct genetic clusters, although hybridization was occasionally detected. (*ii*) By contrast, in nearly half of the taxonomical species investigated, we found several sympatric genetic clusters, indicating that cryptic species are not uncommon. (*iii*) Interestingly, a re-examination of morphological traits associated to each genetic cluster can reveal diagnostic characters, so that taxonomy can greatly benefit from population genetics approaches to resolve species complexes. (*iv*) Finally, we found that markers from the chloroplast genome were not always reliable to distinguish closely related species, limiting the usefulness of plastid-based DNA barcodes. **Significance:** According to our results the number of African tropical tree species might in reality be underestimated by a two-fold factor due to (near) cryptic species.

The phylogenetic structure of plant assemblages in tropical Africa: from local community to biogeographical scales

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Background: Phylogenies can help understand the processes governing species assembly in natural communities because they convey information on species shared history and evolution. Here, plant phylogenies based on *rbcL* and *matK* DNA barcodes were used to investigate the phylogenetic structure of plant communities at two contrasting scales: (*i*) a 50 ha plot in western Cameroon, and (*ii*) the different floristic regions of tropical Africa. Plant distribution data came from (*i*) a systematic census of all trees (~300 000 stems from ~450 species), and (*ii*) a newly assembled large floristic database for Tropical Africa, called RAINBIO (~600 000 occurrence points from ~25 000 plant species). For each dataset, we investigate in particular species turnover and lineage turnover to obtain insights on assembly rules. **Results:** Within the 50 ha plot, a general pattern of phylogenetic clustering occurred at all the scales investigated, and phylogenetic turnover in space was correlated with topographic habitat differentiation. These results suggest that local environmental conditions tend to favor the establishment of phylogenetically related species, and we did not find evidence of processes favoring the local assembly of less related species that would be more complementary in terms of niche partitioning. At the continental scale, species and lineage turnover between floristic units depends both on geographic distance and on ecological contrast, but the turnover of lineages better correlates with ecological distances than with spatial distances. Species turnover and lineage turnover can therefore provide complementary information to define vegetation units that highlight biogeographic isolation and ecological differentiation, respectively. **Significance:** Plant phylogenies offer new perspectives to understand the origin of floristic assemblages. To this end, new theoretical work is probably needed to better establish the links between patterns and process in order to identify the best ways to integrate community and phylogenetic data.

DNA barcodes from old museum specimens for completion of DNA libraries and for realizing difficult XXL-revisions (Lepidoptera, Geometridae)

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Background: Next-generation sequencing (NGS)-based techniques and Sanger barcoding with mini-primers provide a robust basis for taxonomic revisions by generating barcode sequences for old type specimens and other important museum vouchers. **Results:** So far, 3313 German lepidopteran species have been DNA barcoded, covering 88% of the national fauna. The gaps are now being filled in the second phase of the German Barcode of Life (GBOL) project by submitting tissues from old museum specimens (including European type specimens) to NGS protocols. So far, COI sequences were generated for 197 European Lepidoptera based on vouchers older than 80 years and for 292 lepidopteran type specimens from Europe. For taxonomy, we

tested that approach for one of the most difficult geometrid groups, the genus *Prasinocyma* Warren, 1897, for which most collections do not offer species-level identifications at all. The world catalogue of geometrid moth names (Scoble 1999) lists 94 valid species for the Afrotropical region. We were able to provide a solid basis for the described biodiversity by generating DNA barcodes, genitalia dissections, and adult photographs for most existing type specimens. After submitting another 800 African Prasinocyma to DNA barcoding, more than 240 genetic clusters (Barcode Index Numbers (BINs)) were found, apparently including many undescribed species. **Significance:** For the investigation of type specimens in taxonomic revisions we plea for a minimal invasive approach involving DNA barcoding with tissue recovery and morphological examination of genitalia by three-dimensional microCT scanning. We were able to show that non-destructive microCT technology provides similar information as the highly invasive "conventional" genitalia dissections.

DNA barcoding the San Diego County Plant Atlas using a herbarium synoptic collection from a global biodiversity hotspot

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Background: The San Diego Barcode of Life (SDBOL) is a unique, regionally-led initiative centered in an advanced life sciences research and innovation hub (sandiegobarcodeoflife.org). The San Diego County Plant Atlas (www.sdplantatlas.org) engaged 500 citizen scientists in a 5-year project to document the floristic diversity and species distribution of this global biodiversity hotspot, located in the California Floristic Province. The 55 000 specimens collected for the San Diego Natural History Museum led to the discovery of over 300 new county records, 10 new state records, and two new plant taxa, increasing knowledge of local floristic diversity. The Plant Atlas included the expert identification and vouchering of new specimens and retrospective geo-referencing of historical San Diego specimens. To enrich the scientific value of the collection even further, SDBOL, funded by local medical science firm ResMed Inc., initiated a DNA barcode reference library for this taxonomically rich region by sampling a synoptic collection of herbarium specimens, in conjunction with high-resolution image scanning. **Results:** Three loci (*rbcL*, ITS2, *matK*) were sequenced from 3379 sampled specimens, representing 2747 species. A total of 6152 sequences resulted in 2450 successful specimen sequences (72.5%) for *rbcL*, 1965 (58.1%) for ITS2, and 1737 (51.4%) for *matK*. **Significance:** SDBOL achieved the first complete DNA barcoding of a regional flora in a global biodiversity hotspot. Barcoding the San Diego County Plant Atlas reference library added precedent-setting scientific value to this significant collection. Collaborations initiated by SDBOL have contributed over 50 000 barcode sequences to the Barcode of Life Data System (BOLD), contributing to the creation of a comprehensive San Diego Barcode of Life reference library.

Evaluation of eDNA metabarcoding for assessment of benthic impacts of salmon farms compared to traditional morpho-taxonomic and physico-chemical methods

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Background: Aquaculture production of marine finfish has been increasing rapidly in past decades and plays an important role in the economy and human food supply. The sustainable development of finfish aquaculture requires effective monitoring methods to assess potential impacts on the benthic environment. To date, traditional benthic monitoring methods typically include biochemical (porewater sulfide, dissolved oxygen, and organic content) and biological attributes (macrofaunal diversity). Traditional measurement of benthic species diversity using morpho-taxonomic methods requires highly specialized expertise and is cost- and time-intensive; hence it is not practicable for routine industry monitoring. Environmental DNA (eDNA) metabarcoding is a novel, cost-effective, and rapid method of assessing biodiversity in environmental samples, and thus it has high potential to assist in the assessment of benthic impacts of fish farms. **Results:** Here, we present results from 84 sediment samples collected along organic enrichment gradients (based on porewater sulfide and organic content) in the dominant current direction at two fish farms in British Columbia, Canada. We employed an eDNA metabarcoding approach using three markers targeting foraminifera, meiofauna, and eukaryotes to identify eDNA-based bioindicators of benthic organic enrichment. To do this, we characterized benthic biodiversity using traditional morpho-taxonomy of benthic macrofauna and measured a variety of physico-chemical parameters of sediments for comparison with eDNA data. **Significance:** Results presented here are findings to date within a larger study that will analyze 252 sediment samples at six farms with different sediment types and organic contents in British Columbia to firmly establish relationships in this geographic region between eDNA data and more traditional morpho-taxonomic and physico-chemical methods. The goal is to evaluate the performance of eDNA metabarcoding for routine benthic monitoring at soft-bottomed finfish aquaculture sites. Findings will be used to inform the national standard for regulatory monitoring of benthic impacts in Canada.

Revealing and reading life through deep barcoding

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The DNA barcode community has been wading through the genomic shallows, rarely gathering more than a few thousand records from a particular place or group of organisms. We must learn to swim if we are going to assemble the 500 million records needed to parameterize a barcode reference library for all animal species. Add a similar number to extend coverage to plants, fungi, and protists. A billion barcodes—that's serious swimming. However, it's not enough to enable biodiversity science to track the shifting distributions and abundances of species in space and time. That's going to require hundreds of billions if not trillions of barcodes. This talk will examine some of the insights gained from short swims—those examining a million barcodes. It will also consider the prospects for deep diving—how current high-throughput sequencing platforms make it possible to gather reference barcode sequences for pennies and enable species detection in mass samples for a fraction of this amount. Because of these technological advances, the eukaryote barcode library can be completed for a few hundred million dollars, and species surveillance will soon be possible in near-real time. As we know from ocean exploration, deep diving brings unanticipated scientific discoveries. Expect the same as deep barcoding allows us to probe biodiversity to new depths.

Barcoding of Bromeliaceae (Poales)

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Background: The angiosperm family Bromeliaceae comprises 3906 species, almost all of them restricted to the Neotropics (a single spe-

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cies is native to West Africa). The family is characterised by exceptionally high morphological and ecological plasticity and also a very low sequence variability in the up to now studied markers. Most of the species are monocarpic, propagating vegetatively with lateral shoots. In several genera, plants are vegetatively very similar, which makes the determination of bromeliads difficult. Especially in botanical collections, this is a problem, when plants are cultivated several to many years before flowering. Barcoding is a very promising approach to provide fast and cheap determination of bromeliads; however, the observed low genetic variability causes specific problems. **Results:** In the course of a project funded by the German Federal Ministry of Education and Research (BMBF) to improve access and scientific use of living collections in Botanic Gardens, a number of markers were tested for their suitability for barcoding (nuclear: Agt1, ETS, PHYC; plastid: *matK*, *ycf1*). Taxonomically comprehensive and reliably determined sampling was provided from Botanic Gardens and especially from the private collection of one of the authors (E.L.). Especially, the highly variable nuclear marker Agt1 was identified as a potential genetic barcode suitable for identification. **Significance:** The barcoding approach will improve the availability of bromeliad collections in Botanic Gardens.

The importance of adaptive variation in geographic range change under climate change

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We know from past climatic shifts that species track changing conditions through migration. Paleo records of past migration, however, tell us little about how the genetic structure of species affected, and was affected by, changing conditions. In this presentation, I will share a perspective on the potential importance of local adaptation in determining the geographic response of species to modern climate change, and I will illustrate how we can use common garden experiments and genomic techniques to gain insights into current adaptive population variation for climate. I also will discuss how recent climatic changes have molded a hybrid zone by changing the geographic location of traits associated with climatic tolerance and seasonality. These studies were performed with butterflies, but the concepts and processes can apply to many other species. Understanding such processes will play a key role in how successfully we can anticipate and manage changes in biodiversity over the coming decades.

Assessing DNA barcodes as an aid for species identification of ticks (Ixodida) in the United Kingdom

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Background: The family Ixodidae comprises of 14 genera and nearly 702 described tick species worldwide. Many species are important zoonotic disease vectors due to their capacity to transmit numerous bacterial, viral, and protozooan pathogens. The morphotaxonomy of ticks is challenging due to the shared morphological traits between species at all developmental stages including larval, nymphal, and adult. In the present study, we evaluated the utility of COI DNA barcoding approach to identify ticks in the United Kingdom (UK). In addition, we assessed the barcode variability within and between species to reveal any hidden diversity within morphospecies. **Results:** This study has constructed a reference library of DNA barcodes for six ticks species in UK, one exotic species (*Rhipicephalus sanguineus*), and another species (*Dermacentor marginatus*) that is morphologically very similar to *D. reticulatus*. In all cases, individuals of the same morphospecies grouped together in the neighbour-joining analysis. Levels of genetic divergence were variable across taxa. For example, while conspecific individuals collected from a single locality often exhibited zero or low divergence, geographically separated individuals exhibited higher divergence. The overall genetic divergence within species averaged 1.6% (ranging from 0% to 0.5% for UK tick species), while divergence in the exotic species, *R. sanguineus* (globally distributed), reached 5.08%. The interspecific divergence averaged 26% (range 15.9%–30%). Barcode sharing was not found in the dataset, and all Barcode Index Numbers (BINs) agreed with the assigned morphological species. **Significance:** This is the first study to compile a DNA barcode reference library to provide species-level identifications for UK ticks. It also reports the first full COI DNA barcode for *Carios vespertilionis* (the short-legged bat tick) in the UK. The study highlights the merit of further investigation to obtain COI DNA barcoding data on UK ticks.

Impacts of forestry on spider (Araneae) diversity, abundance, and community structure

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Background: Arthropods are a major component of forest biodiversity, critical to nutrient cycling and transfer. Certain arthropods, such as spiders, are sensitive to changes in environmental conditions and have been used as ecological indicators to monitor the effects of disturbance. This study aims to understand the effect of forestry on spider diversity and assemblages in Algonquin Provincial Park, Ontario, Canada. We used DNA barcode-derived estimates of diversity (using Barcode Index Numbers (BINs) as proxies for species) to alleviate the taxonomic impediment (difficulty in accurate identification of immature and damaged specimens) in order to compare differences in diversity and community structure between sites with different disturbance histories. Furthermore, we recorded abiotic factors such as temperature and habitat complexity in an attempt to explore the underlying mechanism that may be driving differences between disturbed communities. **Results:** Our data suggest that cut forests were characterized by a higher maximum temperature, lower habitat complexity, lower phylogenetic diversity, and were more phylogenetically clustered than uncut forest. We observed trends of decreasing similarity over distance, indicating that regional effects over short distances (<150 km) can be a significant factor affecting community composition. We did not find a difference in guild composition between treatments. We found a change in species composition between treatments, with 28 species (20%) unique to the uncut treatment, 53 (38%) species unique to the cut sites, and 58 (42%) cosmopolitan species. This suggests a possible decoupling between functional diversity with phylogenetic and taxonomic diversity. **Significance:** Our findings suggest temperature might be a driving force for spider phylogenetic structure, and regional effects may play a larger role than previously expected. Caution should be exercised when attempting to use phylogenetic diversity in conservation as a proxy for taxonomic or functional diversity when assessing the impact of anthropogenic disturbance on arthropod diversity.

DNA barcoding plants using hybrid capture

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DNA barcoding using Sanger sequencing is widely used for plant identification, taxon discovery, and diversity studies (e.g., spatial phylogenetic analyses). Metabarcoding studies using high-throughput next-generation sequencing platforms are also now well established, and various protocols are in place for recovering standard barcodes for specimen-based studies using new sequencing technologies. A remaining limitation is that many plant species are simply not distinguishable by plastid or ribosomal barcode markers. In this talk, I will consider the nature of differences among plant species and explore

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mechanistically why barcode markers are likely to be shared among taxa. I will then review the implications of recent phylogenetic studies using hybrid capture for targeting multiple nuclear loci for telling species apart. Combined, I will use this information to evaluate the types of situation where hybrid capture methods may be suitable for high-throughput species identification and delimitation.

Metabarcoding using 18S rDNA reveals unprecedented eukaryotic gut microbiome diversity governed by conserved ecological processes in a non-human primate

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Background: The majority of eukaryotes have been suggested to live on or in other organisms, but the diversity and ecology of these symbiotic eukaryotes remains consummately uncharacterized, particularly in contrast to prokaryotic microbiomes. Nonetheless, the keystone roles played by eukaryotes in free-living systems, and the ubiquity of parasitism, commensalism, and mutualism, suggest that symbiotic eukaryotes may be important components of host-associated communities. **Results:** Here, we utilize an Illumina sequencing approach based on 18S rDNA to characterize eukaryotic diversity within the feces of wild long-tailed macaques (*Macaca fascicularis*) on two islands in southeast Asia: Singapore and Bali, Indonesia. We report substantially higher levels of eukaryotic diversity than previously reported and comparable to free-living ecosystems. Moreover, taxa representing all eukaryotic supergroups occurred universally across samples. While several groupings of parasitological significance were reliably detected, we also uncover a vast array of organisms that are not associated with pathogenicity and which could perform important functional roles within their host. Resident eukaryote community composition was assessed with regard to functional guilds grouped according to trophic strategy. Symbionts utilizing grazing, predatory, and intracellular trophic strategies were seen in all samples. Taxa composition was fluid, but co-varied in accordance with the trophic relationships expected between these guilds. **Significance:** Overall, our findings suggest that vertebrates host an enormous component of undescribed eukaryotic diversity that is likely governed by the same ecological principles as free-living systems, and highlight a role for parasitism, commensalism, and mutualism as recurrent and dominant life history strategies within eukaryotic ecology and evolution.

Influence of ectomycorrhizal trees on the fungal communities in West African woodlands

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This study took place in northern Benin, which aims to (*i*) determine the influence of the presence/absence of ectomycorrhizal forest trees on fungal communities and (*ii*) determine the influence of the dendrometric parameters (density and basal area) of ectomycorrhizal (EcM) forest trees on the abundance and dominance of EcM mushrooms. Nine permanent plots of 2500 m² were installed in three different phytocenoses, dominated each by *Isoberlinia doka* (V1), *Isoberlinia tomentosa* (V2), and *Uapaca togoensis* (V3). Mycological surveys were conducted at a frequency of two times/ placeau/week during the 17-week period. Representative specimens of each species were selected, dried, and preserved in order to constitute reference material. We used DNA barcoding to identify different species that we collected. For this study, the floristic variables used are the basal area, the relative contribution of each target EcM tree to total basal area of the sub-plots and plots, and the density of EcM trees while mycological variables considered include the number of fruit bodies and the fresh biomass per species/subplot/week. These data were analysed in R using statistical analysis such as the Mantel test and the Canonical Correlation Analysis. We recorded a total of 110 species. Analyses revealed that there is no relationship between the fungal community and the mushroom community of EcM trees in the different plots (p=0.15). However, the influence of the neighbouring plots on the diversity of fungal communities is highlighted (p=0.02), except as may be explained by spatial autocorrelation between sub-plots and plots. The study showed that there is a relationship between the basal area of trees and the (*i*) number of carpophore (p=0.01), (*ii*) the fresh biomass EcM mushrooms (p=0.014), and (*iii*) a strong correlation between the fresh mushrooms EcM biomass and density of trees EcM (p=0.01).

rDNA nucleotide-based phylogeny 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. It has been demonstrated that misidentification, mostly of cryptic species, has led to the death of many people, whilst traditional taxonomical methods hamper our ability to assess global diversity of fungi. To get a clear picture of fungal diversity and community phylogenetics, systematic sampling of fruit bodies of ectomycorrhizal fungi was carried out in species-rich ecosystems of Benin. We recorded a total of 110 morphological species in 33 genera. 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, cleaning with chloroform, and alcohol precipitation. The internal transcribed spacer (ITS) region of the rDNA was amplified by PCR using, variously, the primer pairs ITS1-F/ITS4, ITS1-F/ITS4-B, or ITS1/LB-w, and sequenced using the Sanger method. **Results:** We generated a total of 116 sequences sorted into Russulaceae (33 sequences), Amanataceae (43 sequences), Boletaceae (37 sequences), and Cortinariaceae (3 sequences). Similar sequences were downloaded from GenBank to generate a dataset of 3304 sequences. In the present talk, we will showcase the placement of our sequences within the global phylogenetic context, whilst the consistency of traditional delimitation of species and sections within core genera will be tested phylogenetically. **Significance:** We expect to depict strong phylogenetic proof to support the description of numerous putative new species and to support delimitations within cryptic taxa. Our investigations will increase our understanding of species limits within taxonomically complex genera. Results from the present study will nourish the Fungi DNA Centre under construction at the University of Parakou.

Vascular plants of the Ewe-Adakplame relic forest in Benin, West Africa

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Palaeoenvironment evolution in West Africa has shown that tropical forests were shrunken to small refugial areas and are since in a state of constant isolation. In Benin, the Ewe-Adakplame relic forest (EARF) is among these remnant forests showing rather insular characteristics within the Dahomey gap savannas. This forest is also suspected to have survived ecosystem deterioration since the

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early mid-Holocene. However, vascular plant lists are not comprehensive, and EARF is not even seen yet as conservation priority. This study provides floristic composition of EARF and description on life forms and chorology of vascular plants. We recorded 180 species belonging to 58 families and 142 genera. Of these, the family Rubiaceae was the most represented with 20 species; followed by Fabaceae (15); Apocynaceae (8); Sapindaceae (8); Annonaceae and Sterculiaceae (7); Euphorbiaceae (6); Capparidaceae, Dioscoraceae, and Ulmaceae (5). Twenty-five families were represented by one species each. Only the genus *Dioscorea* is represented by five species. The life forms and chorology showed that important taxa corresponded perfectly with phanerophytes of the Guineo–Congolian region. Species richness estimation divergences using Bootstrap, Chao, Jacknife1, and Jacknife2 were 200.52 ± 9.2808; 217.62 ± 14.5972, 224.16 ± 15.3725, and 242.67, respectively. This demonstrated that the correct richness lays somewhere within this range. *Nesogordonia papaverifera*, *Mansonia altissima*, *Drypetes aframensis*, and probably *Englerophytum oblanceolatum* are among recorded species found nowhere else in Benin. EARF has an exceptionally rich flora with a high patrimonial significance. The uniqueness of this Guineo–Congolian vegetation in a savanna-dominated area of Benin prompts for adequate conservation involving modern and traditional methods and considering multifunctionality of community lands, conservation, and economic challenges. The study also stressed the urgency to highlight the history linking men and EARF over millennia in order to provide new arguments for engaging scientists and developers into sustainable conservation actions.

Combining DNA barcoding and ecological niche modelling to strengthen control and management plans of invasive plants of freshwater systems in South Africa

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Background: Controlling or eradicating invasive plants, especially those of fresh water systems, is costly to the South African government. In addition, the success of these operations is mixed because aquatic invasive plants often spread very rapidly before they are detected or before control measures are taken. This mixed outcome is further compounded not only by difficulties linked to quick and accurate species identification but also by changing climate. The objective of this study is to facilitate rapid species identification and identify areas climatically suitable for future invasion to facilitate pre-emptive actions. To this end, we tested three DNA markers (*rbcLa*, *matK*, and *psbA-trnH*) as potential DNA barcodes for invasive plants of freshwaters and applied ecological niche modeling to identify potentially suitable areas of invasion currently and in the future for the distribution of the five most important aquatic invaders in South Africa. **Results:** We found *psbA-trnH* to be a suitable and reliable DNA barcode for the identification of invasive freshwater species in South Africa. In addition, our models indicated that 38% of all South Africa's dams occur in areas climatically suitable to the invasion by these aquatic species. However, our model also indicated contrasting effects of climate change on the future distribution ranges of invasive species. **Significance:** Our study demonstrates not only the utility of DNA barcoding in implementing control measures, but also provides ways of prioritizing preemptive control efforts.

So long suckers: estimating species diversity in a unique but imperiled Amazon catfish fauna

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The proliferation of hydroelectric dams in the Amazon basin may have profound effects on aquatic biodiversity. Suckermouth catfishes (Loricariidae: Ancistrini) are a mega-diverse but an understudied group, important in nutrient cycling of Neotropical rivers. Here, we examine the fauna of the Xingu and Tapajós, two rivers under imminent threat from large dam projects. We used standard DNA barcoding together with four analytical methods (locMinBoot, GMYC, bGMYC, mPTP), here adapted to incorporate phylogenetic uncertainty and provide a confidence interval around the number of species estimated from the molecular data. A total of 27 named species were found in our sample of >500 individuals, and 59 distinct morphotypes also recorded. Molecular estimates were similar to one another, comprising between median 31 species (mPTP) and median 42 species (GMYC). Overall, our results indicate that ancistrin loricariid diversity is much higher in these rivers than the number of named species suggests, but also that molecular diversity estimates are substantially lower than those of the morphological analyses. This discrepancy can be explained by the young age of many of the species-groups, being inseparable with barcode data, and likely comprise ongoing speciation events, highlighting the unique status of these threatened sites as possible "species pumps". Despite the inferred underestimate of diversity, DNA barcodes highlighted previously unrecognised within-drainage endemicity and provides a standardised sampling methodology for baseline environmental impact assessments of large infrastructure projects.

Intraspecific DNA barcode divergence versus cryptic diversity: lessons from a large-scale survey of Lepidoptera in the Alps

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Background: The European Alps are a hotspot for Lepidoptera diversity on this continent. With about 5500 species, this area hosts more than 50% of the European fauna, although it represents just 2% of the land. The "Lepidoptera of the Alps" campaign is assembling a DNA barcode reference library for this region as a contribution to the goals of the International Barcode of Life Project (iBOL). A regional subproject is testing patterns of intraspecific barcode divergence in the biogeographical suture zone between the eastern Alps of Austria and Italy. The main ridge of the Alps is suspected to have acted as a major topographic barrier during glacial periods, creating a favourable situation for genetic diversification. **Results:** We present a DNA barcode library for 2565 Lepidoptera species (70 families) based on the analysis of more than 10 000 specimens. Species differ from their nearest neighbour by an average minimum distance of 6.38%, while mean intraspecific divergence is only 0.45%. Unequivocal identification was determined from Barcode Index Numbers (BINs) for 2442 species, representing 95.2% of all species, while barcode-sharing or overlap was found in 84 species. Deep intraspecific splits (>3%) were detected in 72 species, indicating cryptic diversity. In addition, nine new species were described. Fifty of the 1835 species from the southern part of the research area were represented by unique barcode clusters with >0.5% divergence from their conspecifics in the North despite their geographical proximity. These cases indicate that the major ridge of the Alps acted as an important phylogeographic barrier during the Pleistocene. **Significance:** Our large DNA barcode library confirms the utility of this method for species identification. However, it also revealed a surprisingly high inci-

dence of both cryptic diversity and phylogeographic splits in this well-investigated area. Moreover, the results from this study provide important context for several international co-operative projects, particularly on the arctic–alpine fauna.

Cryptic, but not that much: Mediterranean brackishwater *Gammarus* (Crustacea: Amphipoda) moderately follow trend unraveled in freshwater congeners

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Background: Amphipods of the genus *Gammarus* are a salient component of communities in European inland and coastal, marine, and brackish waters of the North Atlantic, the Mediterranean, and the Black Sea. Exceptional levels of cryptic diversity have been revealed for several widespread freshwater species of *Gammarus* in Europe. No comprehensive assessment has yet been made for brackishwater counterparts, such as *Gammarus aequicauda* and *G. insensibilis*, which are among the most frequently recorded members of the so-called "*G. locusta* group" in the Mediterranean and in the Black Sea. Here, we probe the diversity of these morphospecies, examining the partitioning of COI-5P DNA barcodes across multiple populations along their distribution range. **Results:** We generated and compiled 510 bp COI-5P barcodes for a collection of 212 individuals from 63 locations of *G. aequicauda*, *G. insensibilis*, *G. crinicornis*, and *G. locusta*, sampled along the European Atlantic coast between Germany and Portugal, and in the Mediterranean including the Black Sea. All five molecular operational taxonomic unit (MOTU) delimitation methods applied revealed deep divergence between Black Sea, Mediterranean, and Atlantic populations in both *G. aequicauda* and *G. insensibilis*. There were 4–8 distinct MOTUs delimited for *G. aequicauda* (3.0%–14.0% K2P) and 3–4 MOTUs for *G. insensibilis* (5.0%–14.0% K2P). No sympatric MOTUs were detected, and both species displayed several MOTUs within the Mediterranean but single MOTUs in the Atlantic or within the Black Sea. **Significance:** Our results indicate a predisposition for cryptic diversity within Mediterranean brackishwater *Gammarus*, similar to that observed for freshwater counterparts, although much more moderate. Nevertheless, even considering only the most conservative MOTU numbers, confirmation of these findings would translate into the triple and quadruple of known species, respectively. Implications are major in light of the ecological relevance of these species and their extensive use as bioindicators and in ecotoxicological studies.

Barcoding ikhathazo (*Alepidea*, Apiaceae): methods to quantify and monitor trade

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Background: The genus *Alepidea* comprises ~28 species, almost all of which are endemic to southern Africa. The rhizomes of some of the species are highly sought after herbal remedies, ranking in the top five medicinally traded plants within the provinces of KwaZulu-Natal and Mpumalanga and top 20 in the eastern Cape. Rhizomes are used for respiratory and abdominal complaints and sold at muthi markets as "ikhathazo" (isiZulu), "lesoko" (Sesotho), and "iqwili" (isiXhosa). Harvesting rhizomes for urban trade has become a lucrative form of income, resulting in sharp declines in wild populations. Literature traditionally referred to *A. amatymbica* as being traded, yet two other species, *A. cordifolia* and the highly localized *A. macowanii* (and possibly others), are also reportedly harvested. As the rhizomes are separated from the diagnostic plant parts at informal medicine markets, accurate identification and monitoring of the species being traded is usually an impossible task. **Results:** We tested the potential for fingerprinting the most likely traded species using novel hyperspectral imaging (HSI), liquid chromatography (LC-MS), and DNA barcoding techniques. All three techniques showed potential as an effective and simple tool for identification of the rhizomes of the traded species, especially from possible substitutes. All three species from the *A. amatymbica* species group were confirmed to be traded in the informal medicine markets. *Alepidea cordifolia* (rather than *A. amatymbica*) was found to be the most popular traded species. None of the commercial samples studied showed significant amounts of possible substitution with the widespread *A. peduncularis* or *A. setifera*. **Significance:** These methods reveal promise for quantifying and monitoring the trade of "ikhathazo" and to inform conservation policies for safeguarding these species in the wild.

Barcoding and infection dynamics of intermediate snail hosts of human and livestock schistosome flukes

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Background: The epidemiology of schistosomiasis in Senegal is very dynamic. While *Schistosoma mansoni* was the dominant parasite at the onset of the epidemic, the urinary species, *S. haematobium*, was mostly absent. Nowadays this pattern is almost completely reversed. In addition, molecular analyses revealed that children were infected with a hybrid between *S. haematobium* and *S. bovis*, the latter being a livestock parasite. This species uses a different snail host to complete its life cycle. It is not known, however, which snail species is used by the hybrid. If it is able to use the same host as *S. bovis*, it could explain the rise in urinary schistosomiasis because this snail species is very abundant across northern Senegal. To test this we sampled the main snail intermediate host species in the lower and middle delta of the Senegal River Basin (2012–2014). We barcoded the snails by sequencing or RFLP analysis of partial cytochrome *c* oxidase 1 (COX1) and tested each snail for schistosome infection using a diagnostic PCR. **Results:** The most dominant snail species was *Bulinus truncatus*, the host of *S. bovis*, followed by *B. globosus*, the main host of *S. haematobium*. The former was exclusively infected by pure *S. bovis* parasites, with the exception of the snails from the middle delta, while the latter was infected with *S. haematobium* and with hybrid parasites. The distribution of both species was heterogeneous along the river basin, as was the distribution of the hybrids in children obtained during a previous study. **Significance:** These results show that *B. globosus* is the most important snail species for human schistosomiasis in the lower delta. However, *B. truncatus* of the middle delta appears susceptible to both human and veterinary schistosome species. If this species manages to colonize the lower delta, it might strongly impact schistosomiasis epidemiology.

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Applying the latest next-generation sequencing technology – MinIon – to DNA barcoding based fungal identification

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Background: Over the past decade, enormous progress has been made in the sequencing technology. There has been a fundamental shift away from conventional DNA sequencing introduced by Sanger and considered as the first generation sequencing technology to newer methods referred to as next-generation sequencing (NGS). The next promising sequencing platform is the MinION/Oxford Nanopore, which is the first commercially available palm-sized sequencer running on a personal computer using nanopore technology, representing a significant step forward from existing technologies. The device is capable of generating high-throughput, ultra-long sequence reads in real time at relatively low cost, highlighting its potential utility in rapid clinical diagnostics, including pathogen identification. In the current study, we tested and optimized the MinION technology for fungi to assess its promising application into mycological disease diagnostics. Besides pathogen ID, it can also be used in parallel to type strains for epidemiological purposes or detect genes responsible for antifungal resistance. **Results:** We tested the applicability of using the MinION for fungal ID by generating sequences from DNA extracted from pure fungal culture and clinical specimens. MinION sequencing comprehensively identified pathogens in connection to quality-controlled databases. However, better bioinformatics pipeline optimization for fungi and a simpler library preparation is needed to ensure fast and reliable detection and typing of fungal pathogens. One main drawback of the technology in its widespread clinical setting is its current high cost per sample (1000 USD). **Significance:** Currently, there is a growing interest in rapid metagenomic-sequencing-based diagnosis directly from clinical samples without prior culturing. The Nanopore technology is one of the most promising devices after resolving the technical issues and associated high cost.

Eco friendly error-free workflows for high-throughput DNA barcoding

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Background: DNA extraction is a critical stage in any DNA barcoding workflow, and any errors may lead to irrecoverable sample loss or a compromised audit trail. Since 2006, the Canadian Centre for DNA Barcoding (CCDB) has been deploying high-throughput semi-automated protocols for DNA extraction from diverse groups of organisms on Biomek FX liquid handlers, with a capacity to process 1 million specimens a year. These protocols utilize bind-wash-elute steps on glass fibre membrane plates. While most of the workflow is automated, the elution stage and subsequent PCR setup require manual intervention, introducing risk of human errors. Moreover, this protocol produces 4–5 L of toxic guanidinium thiocyanate (GuSCN) waste per day of extraction. **Results:** To overcome these limitations, we developed a fully automated workflow utilizing SPRI protocol with magnetic beads in 384-well plates. We validated this method on 1520 specimens representing four types of insect material: fresh Malaise trap, old Malaise trap (stored for a year), dry pinned insects, and whole vouchers. Samples were first assembled in 96-well plates and then consolidated into four 384-well plates to evaluate four lysis buffer combinations: guanidinium chloride (GuHCl), GuHCl + Proteinase K, GuSCN, GuSCN + ProteinaseK. The GuHCl-based protocols for lysis and binding resulted in 1.7× higher average raw data intensity signal in Sanger sequencing, compared to GuSCN lysis buffer. While Proteinase K treatment was critical for the improvement of sequence recovery from dry pinned insects and whole vouchers in both buffer systems, it can be omitted for Malaise trap material. Consolidation of four 96-well lysis plates into a 384-well plate for DNA extraction is guided by a fly-by barcode reader acquiring information from LIMS, followed by PCR setup before the plates are sealed. **Significance:** The proposed error-free workflow is five times cheaper and produces 30 times less toxic waste compared to existing glass fibre protocols without loss in processing capacity or DNA quality.

Disentangling causes of DNA barcode sharing: genomic evidence for species integrity and historical introgression in wolf spiders

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Background: DNA barcode sharing is a widespread phenomenon among various groups of organisms. It can result from operational causes (such as oversplitting of species, misidentifications, contamination, NUMTs) or two different biological causes: (*i*) incomplete lineage sorting and (*ii*) introgression. We studied the cases of DNA barcode sharing within two groups of wolf spiders from Finland with help of ddRAD sequencing data. **Results:** Freshly collected samples of *Alopecosa aculeata*, *A. taeniata*, and *Pardosa pullata* group of species were barcoded and again showed insufficient differences in their mtDNA to distinguish between them. On the contrary, genome-scale sequencing data supported the status of each species as a separate lineage. Simultaneously, we detected widespread introgression that is likely to be the main cause of DNA barcode sharing among studied taxa. Endosymbionts (*Wolbachia*, *Rickettsia*, and *Spiroplasma*) were detected in both groups of spiders, but they were scarce and we could not prove their role in shaping mtDNA distribution in focal species. Based on our tests, incomplete lineage sorting could be discarded, and operational causes could be ruled out, as the results of genomic data analysis suggest the valid status of studied species. **Significance:** To our knowledge, this study is the first applying genomic data to investigate DNA barcode sharing. By providing a genome-wide overview and large amounts of genomic data, ddRADseq shows an efficient way to study taxonomy of problematic groups with insight into underlying evolutionary processes, thus providing a sound basis for species delimitation. In addition, we attempted to unify the term "DNA barcode sharing" and hope for its widespread application to appropriate cases in DNA barcoding.

The identification of the fall armyworm in South Africa: a matter of quarantine importance

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Background: The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), originates from the tropical regions of the United States of America, Argentina, and Caribbean Islands. It is highly polyphagous and a serious pest of maize. The first reports of outbreaks of the FAW in Africa came from West and Central African countries, but they were initially attributed to indigenous *Spodoptera* spp. During late 2016, the first unconfirmed reports of armyworm damage to maize were received from Zambia and Zimbabwe, followed by reports of an unknown armyworm, damaging maize plants on farms in the Limpopo and North West provinces of South Africa. In this study, we used both morphological and genetic techniques to provide rapid and accurate identification of species for decision making. **Results:** Male moth specimens were morphologically identified as *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). The identifications were confirmed by sequences generated for the *COX1* barcoding gene. **Significance:** The FAW is classified as an A1 quarantine pest on the list of the European and

Mediterranean Plant Protection Organisation (EPPO), and it is a quarantine pest in South Africa. Accurate identifications are essential as part of an integrated approach to the control of this pest by the South African Department of Agriculture, Forestry and Fisheries.

The National Collection of Fungi: a database for phytopathogenic and soilborne fungi from South Africa

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Background: The Mycology Unit of the Biosystematics Division, ARC-Plant Protection Research, serves as the custodian of South Africa's National Collections of Fungi (SANCF). SANCF houses two major collections, as well as several smaller ones. The live culture collection (PPRI) presently houses 22 000 isolates and is affiliated to the World Federation of Culture Collections. The fungarium collection (PREM) traces its origin back 111 years and currently accommodates more than 61 000 specimens, including ~3000 type specimens. These specimens represent not only South African, but also African fungal biodiversity. **Results:** DNA barcodes have been generated for 2537 fungal strains in the PPRI collection. These represent 93 genera and 150 species. Different gene regions were selected depending on the most appropriate gene regions identified for the specific genera. The original genus identifications were based on morphological identifications. **Significance:** The mobilization of collection data associated with PREM specimen holdings and literature, as well as the generation of DNA barcodes for all species represented in the PPRI collection, will enable the establishment of a portal for South African phytopathogenic and soil-borne fungi. These data portals will facilitate accurate fungal identification by quarantine officials, scientists, and citizen scientists, which is essential for food security and trade in agricultural and forestry produce.

DNA barcoding of freshwater fishes from the northern Western Ghats of India

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Background: DNA barcoding has now become a widely adopted tool for taxonomic delineation and species recognition. DNA barcodes, when analyzed by using relevant techniques, provide an imperative approach towards validation of prevailing taxa and putative species (by determining operational taxonomic units (OTUs)). Several supervised methods have recently appeared; some of them have proven their efficiency in taxonomic discrimination and resolution of potentially cryptic species. **Results:** Here, we have assessed 246 DNA barcodes belonging to 81 fish species from the northern Western Ghats of India, using Barcode gap analysis, Barcode Index Number (BIN), Automated Barcode Gap Discovery (ABGD), Poisson Tree Processes (PTP), and General Mixed Yule-coalescent (GMYC). These methods discriminated 97.53%, 93.90%, 95.06%, 93.82%, and 92.59% of species, respectively. However, some of them tended to estimate inconsistent numbers of species, leading to discrepancies between the morphological concept and inference from molecular phylogenetic reconstructions. Therefore, we took a standard approach to recognize those methods that produced consistent results. Three of the five methods revealed three hidden cryptic species complexes in *Monopterus indicus*, *Parambassis ranga*, and *Systomus sarana*. To validate these three genetically diverged species, we used a diagnostic character-based approach along with nine unidentified species through BLOG and WEKAs SMO classifier. Those methods were not able to identify or differentiate these species, which might be due to the limited number of specimens used for the analysis. **Significance:** This is the first effort to generate a DNA barcode reference library of freshwater fishes from the northern part of the Western Ghats of India, one of the world's biodiversity hotspots. These barcodes, when analyzed through a defined workflow, will provide valuable measures to prove the efficiency of molecular species delimitation methods in taxonomic discrimination.

Damn it, Jim, it's a tricorder: a live demonstration of the components of a hand-held, real-time DNA barcoding device

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The Oxford Nanopore Technologies (ONT) MinION is a self-contained, real-time, high-throughput DNA sequencing platform costing less than $1000. About the size of a smartphone, it accepts disposable flow cells and connects directly to a laptop or desktop computer via USB. Now two years out of beta testing, the MinION is routinely used to generate 92%–94% accurate raw sequence reads with amplicon consensus accuracies above 99%. ONT's VolTRAX device performs library preparation automatically, generating MinION-ready DNA with minimal human intervention. The SmidgION—ONT's smallest DNA sequencing device to date—is designed for use with a smartphone. ONT's downstream bioinformatics pipelines can use the Barcode of Life Data System (BOLD) to identify taxa and present results in a user-friendly, tree-based format. These devices and tools, together with parallel advances in DNA extraction and sequence capture, bring the long-wished-for possibility of a hand-held DNA barcoding device—similar to Star Trek's "tricorder"—within reach. During this presentation, we will "make it so" by performing a live, beginning-to-end demonstration of these components to carry out both single-sample barcoding and metabarcoding.

DNA barcoding: grease and glue for integrating conserved wildlands with their respective societies

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In 2003, while we were heavily embedded in the ongoing Victorian "total" inventory of the many thousands of species of caterpillars and their parasitoids in Area de Conservacion Guanacaste (ACG), northwestern Costa Rica, Paul Hebert of Canada's Centre for Biodiversity Genomics introduced us to the technology and concept of DNA barcoding for species identification and discovery. We asked him if he would like many tens of thousands of pinned and ETOH-conserved voucher specimens as a Guinea Pig-White Rat. He replied, "yes, everybody hates me, I have plenty of available work space". However, 400 000+ barcoded ACG insects later in the Barcode of Life Data System (BOLD) and their vouchers in public museums, we would never dream of attempting a bioinventory of any place without at the least (*i*) Sanger sequencing (or a NGS process that generates semi-equal length reads) of samples of everything, (*ii*) BOLD or a BOLD-like process for basic first pass iterative analysis correlated with morphology, behavior, natural history, micro geography, etc., (*iii*) actual or anticipated next step classical taxonomy combined with everything else obtainable (up to and including deep dives into whole genomes), and (*iv*) an internet-based system that passes all this information on to the multiple societies that support and (will) use the outcomes. Our goal is that the local, national, and international societies of all ilks come to accept that this, that, and the other complex conserved tropical wildlands (and oceans) are welcome and legitimate permanent members of their society IN THEIR WILD STATE, to whatever degree is still currently obtainable, given socio-political realities. DNA barcoding is integrative technology for this goal. Examples will be offered as time allows. See <dx.doi.org/10.1139/gen-2016-0005>.

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Hybridization in the species of *Enantia jethys* complex (Lepidoptera, Pieridae)

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Background: With at least 10% of the worldwide species involved, hybridization is common in animal species. Hybridization events have been largely demonstrated in natural butterfly populations. Studies have shown that interspecific gene flow remains important even after speciation, and therefore hybridization and introgression are important factors for the evolution of species as a source of genetic variability. *Enantia* is a butterfly genus of the family Pieridae, and currently contains nine Neotropical species. The *Enantia jethys* complex is a Mesoamerican group composed of three species (*E. jethys*, *E. mazai*, *E. albania*), all of which are sympatric in Mexico. **Results:** We carried out separate and concatenated phylogenetic analyses among Mexican specimens of the above taxa using DNA sequences of three gene markers (COI, RpS5, Wg) and ISSRs. The separate analyses recovered distinct topologies, and all markers had high levels of interspecific gene flow. We found evidence of directional introgression by hybridization. Hybridization always involves *E. albania* with the other two species, but it never occurs between *E. jethys* and *E. mazai*. We also observed that the hybrids can affect the levels of genetic diversity in these species. **Significance:** This study remarks the importance of assessing the presence of hybridization in evolutionary studies of closely related species.

Environmental DNA monitoring detects habitat-specific species assemblages in a marine ecosystem

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Background: The continuing precipitous decline in global biodiversity is driving efforts to find effective and reliable approaches to aid ecosystem conservation and management. New tools to rapidly, and accurately, gather biodiversity data are necessary for informed management. Metabarcoding of environmental DNA (eDNA) allows the simultaneous identification of multiple species from DNA present in environmental samples without biological source material. This technique offers the possibility of monitoring substantial components of biodiversity in a non-invasive, economical, and timely manner. Marine ecosystems could benefit from an eDNA approach, due to their reduced accessibility, cryptic species, and poorly known taxa. However, water movement between habitats through currents and tidal influences could transport DNA from one area to another, leading to false-positive species detection and inaccurate biodiversity data. **Results:** We examined the accuracy of the eDNA monitoring method in a marine setting by comparing the eDNA signal between two neighbouring sites holding different community assemblages (rocky shore vs. sheltered mudflats, <1 km distance). In total, we identified 64 species from three amplicon targets. Taxonomic assignment through BLAST suggested most species present were from the phyla Chordata (37.5%), Arthropoda (17.2%), and Ochrophyta (14.1%). Community structure analysis found a clear difference in the retrieved eDNA signal between our two sites. Habitat preference of detected species showed little evidence of DNA transport between habitats, indicating a local origin of the eDNA signal with a fine spatial resolution. **Significance:** Our results show that eDNA monitoring can detect habitat-specific species assemblage differences in geographically close coastal environments. The lack of evidence for DNA transport through water movement, based on habitat preference validation, proves the accuracy of the eDNA monitoring method in the marine ecosystem. Metabarcoding of eDNA could alleviate the problems of monitoring biodiversity in the marine environment, by accurately and quickly gathering the necessary data for ecosystem conservation and management.

Brachionus paranguensis (Rotifera: Monogononta), a new species from the volcanic maar Rincon de Parangueo, Guanajuato, Mexico

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Background: *Brachionus plicatilis* is a complex of species, most of them not correctly described, dwelling in salt water, and commonly found close to the sea. Some studies have tried to solve the taxonomy of this group by describing some varieties and species as well as using morphological and phylogenetic studies, but most of the species are still waiting for a formal description. **Results:** We found a rotifer from this group in one of the remains of a hypersaline crater lake known as Rincon Parangueo. After analyses of the spines from the shell, the trophi, and a morphological and genetic comparison, including all public sequences of the COI gene (known as DNA barcode) for this complex, we propose a new species: *Brachionus paranguensis* n. sp. This is a close relative to another variety unofficially named *B. nevadensis* from the southern USA. **Significance:** We hope to help detangle the puzzle represented by this complex of species and varieties with this contribution.

Using pollen DNA metabarcoding to investigate the foraging preferences of honey bees

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Background: Honey bees contribute both directly and indirectly to humans; through honey, wax, and propolis, and as the pollinator of both wild and crop plants. The increased rate of honey bee colony loss has caused worldwide concern, caused by the interacting effects of habitat loss and fragmentation, agrochemicals, pests and diseases, and climate change. DNA metabarcoding provides a tool for identifying the pollen in honey, and therefore the plants the honey bees are foraging upon. DNA is amplified using the *rbcL* marker and sequenced on the Illumina MiSeq platform. Using our study site at the National Botanic Garden of Wales, we recorded all plants in flower on a monthly basis, and at the same time sampled honey from hives in the Botanic Garden's apiaries. By using the results of the plant survey compared with the honey DNA metabarcoding results, we can start to build a temporal and spatial picture of honey bee foraging. **Results:** During early season foraging (April and May) 437 genera of plants in flower were recorded in the study site, but only 11% of these were used. Thirty-nine plant taxa were recorded from three hives but only 10 of these at greater than 1%. All three colonies used the same core set of native or near-native plants, typically found in hedgerows and woodlands. The major plants were supplemented with a range of horticultural species. **Significance:** During the spring, honey bees need access to native hedgerows and woodlands to provide major plants for foraging. Gardens provide supplementary flowers that may increase the nutritional diversity of the honey bee diet. Having a detailed understanding of the habitat and foraging requirements of honey bees is

required to fully understand declines and supply guidelines for suitable plants for healthy honey bee colonies.

The use of DNA barcoding to improve the taxonomy of Afrotropical hoverflies (Diptera: Syrphidae)

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Background: Currently, there are about 600 nominal morphospecies of Afrotropical hoverflies (Syrphidae) whose morphological identification are very difficult due to limited recent taxonomic revisions and the lack of comprehensive identification keys. A few years ago, we constructed a reference dataset of ~500 COI barcodes for almost 100 of the more common nominal species from West Africa. The results showed that DNA barcoding is a very helpful tool to identify these species and that it was able to pinpoint those taxonomic groups that are in need of revision. We now have extended this reference barcode dataset (*i*) to get a broader coverage of this group and (*ii*) to cover a larger area of the Afrotropics. **Results:** The current reference database comprises almost 2000 barcodes for almost half the known number of Afrotropical syrphid species. The identification accuracy of this dataset was evaluated with three methods (K2P distance-based, neighbour-joining (NJ) / Maximum Likelihood (ML) analysis, and using SpeciesIdentifier). The identification success estimated using the three methods was high (>95%) and comparable to our previous study. **Significance:** DNA barcoding is a useful alternative identification tool for the Afrotropical Syrphidae. It also improves the taxonomy of Afrotropical hoverflies, and these barcoding efforts have resulted in the start of taxonomic revisions of several of the Afrotropical syrphid genera. Moreover, several other research groups are adding barcodes to the dataset, which will speed up taxonomic revisions even further.

Unravelling relationships in *Tephrosia* and allies (Millettieae, Fabaceae)

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Background: Comprising of some 350 species, *Tephrosia* Pers. is a large genus of legumes mostly confined to Africa, but also extending to North and Central America, Australia, and Asia. Two subgenera are recognized within the genus, i.e., subgenus *Barbistyla* Brummit and subgenus *Tephrosia*. Relationships within the genus and among its close relatives in the Millettieae (*Apurimacia* Harms, *Chadsia* Bojer, *Mundulea* Benth, *Pyranthus* Du Puy & Labat, *Ptycholobium* Harms, and *Requienia* DC., i.e., the *Tephrosia* clade) are not well understood. Therefore, in an attempt to decipher whether the current classification reflects natural relationships and affinities a representative sampling of 236 taxa of the *Tephrosia*-clade from across the world were barcoded (using the core barcoding regions *matK* and *rbcLa*), along with additional markers, i.e., *ycf*1, and the nuclear internal transcribed spacers (ITS). **Results:** The resulting phylogenies indicate that the genus *Tephrosia* is polyphyletic, with *Chadsia*, *Mundulea*, *Ptycholobium*, and *Requienia* embedded within. *Apurimacia* and *Pyranthus* are placed outside of the main *Tephrosia* clade, although the support for this is weak in some analyses. In addition, *Tephrosia* subgenus *Barbistyla* and subgenus *Tephrosia* are not monophyletic. **Significance:** Due to its large size the resolution of the *Tephrosia*-clade represent a great accomplishment for African legume systematics. This study contributes a first large sampling of *Tephrosia* across its vast distribution and a solid phylogenetic hypothesis of relationship within the genera. Implications of the data for the classification of *Tephrosia* will be discussed.

DNA banking and barcoding of endangered tree species in Nigeria

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Background: Technological developments in industrial nations and increasing pressure for agricultural land in developing countries, combined with the exploitation of timber, minerals, and other natural resources, are causing rapid environmental changes. These threaten whole ecosystems as seen by the continuous expansion of deserts in the northern parts of Nigeria, and thus the survival of thousands of plant species are threatened. Today, many tree species face extinction or severe genetic loss, and for most of the endangered tree species, no conservation action has been taken. This project seeks to conserve endangered tree species in Nigeria through DNA banking and DNA barcoding techniques. Our goal is to make a real difference on the ground by contributing to conservation practices by applying the novel biotechnology tool (DNA barcoding) towards the conservation of endangered tree species in Nigeria. **Results:** Initial survey revealed that these threatened tree species include *Albizia* spp., *Antiairs africana*, *Berlinia africana*, *Lovoa trichilioides*, *Prunus africana*, *Vitellaria paradoxa*, *Psedospondias microcarpa*, *Belschemidia* spp., *Tabernomontana* spp., *Pouteria altissima*, *Entandrophragma angolense*, *Pterygota mildbraedii*, *Anthonotha noldeae*, *Isolona deightonii*, *Pouteria altissima*, *Newtonia buchananii*, *Carapa procera*, *Raphia mambillensis*, *Prunus africana*, *Dryptes* spp., *Strombosia* spp., *Polyscias fulva*, *Pterocarpus erinaceous*, *Pterocarpous milbreadii*, and *Macaranga occidentalis*. For the continued survival of these species, it is essential that the diversity of their gene pool be maintained so that they can adapt to the continual small changes in the environment, shifts in climate, changing pressures from predators, disease, competition, etc. **Significance:** This project has the potential to develop considerably the scientific capabilities of Nigeria by ensuring quick identification of tree species, improved control for the movement of species across national borders, opportunity for training of students/researchers, involvement of local researchers in global networks and biodiversity initiatives, and improved national research infrastructure of specimen collections, molecular laboratories, and biodiversity databases.

Keeping up with the Joneses. Resolving large ecological interaction webs on a shoelace

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Quantitative food webs have quickly become a golden standard for ecosystem research. Resolving the strengths of trophic links requires a considerable number of observations of interactions. Thus far, just building a single representative quantitative food web has been challenging, yet the objective of ecologists is clearly to characterize the dynamics of large arrays of interaction webs, and to record how they change in relation to environmental factors or experimental treatments. Recently, the development of molecular methods enabling the identification of dietary items from scat or gut contents to species level has caused a quantum leap in the research on trophic interactions. Yet, for this type of analyses, the cost per data point has easily increased beyond practical amounts. In this talk, we will outline a pipeline specifically designed to process even tens of thousands of samples with a small budget, achieving a per sample material cost of roughly two euros (in Finland inc. tax), including everything from specimen collection to next-generation sequencing analysis of both the specimen and its diet. Major savings are achieved by implementing traditional salt-isopropanol DNA extraction in plate format and by

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superfast preparation of dual-index libraries for massively multiplexed Illumina HiSeq sequencing, while optimizing reaction volumes and circumventing redundant purification steps. Per data point costs would naturally be considerably lower for a system consisting of generalist predators with several detectable prey items per individual. Parasitoid food webs offer practical model system for molecular inference of trophic links. Most importantly, each adult parasitoid originates from a single host, thereby circumventing many methodological challenges associated with mixed sources of template DNA. Hence, we illustrate the utility of the pipeline by applying it to a focal food web consisting of high-arctic lepidopteran herbivores and their parasitoid predators.

Genetic diversity between *Cirrhinus mrigala* from two different habitats (farm and river) on the basis of COI gene sequences

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Genetic identification or DNA barcoding has attracted scientists since the beginning of the 21st century. The shortcomings of traditional morphological methods for identification of fish larvae, eggs, and processed and damaged specimens has demanded an alternative method of taxonomic identification. Partial sequences of the COI gene used as a barcode have proven to be a useful tool for identification of fish species as well as helpful in estimating evolutionary history and genetic diversity. The present study was conducted to investigate the evolutionary history and genetic diversity of *Cirrhinus mrigala* with respect to differences in habitats. Partial sequences of the cytochrome *c* oxidase I (COI) gene from fishes of both habitats were analyzed using MEGA 7 and DnaSP 5. The discovered haplotypes indicated genetic variation among fishes from farm and river. The phylogenetic analysis confirmed the close association and common ancestory of different fishes. The GC content of sequences generated for this study was higher as in other fishes of the family Cyprinidae. All fish populations used in the study showed high haplotype diversity of 0.8571. It is concluded that different fish species belonging to the same genus and family but living in different habitats have genetic diversity among them while sharing a common ancestor.

On resolving the challenges to Neo-Darwinism, molecular phylogenetics, and DNA barcoding arising from current molecular data

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Background: The evidence of the possible impact of genetic introgression on species evolution, the evolutionary fate of taxa, reticulations in phylogenetic trees, and the consistency of the latest molecular genetic data with the main modern paradigm, Neo-Darwinism, are considered. **Results:** The main issues of the report include the following four items: (*i*) A combination of nuclear and mtDNA markers best suits the hybrid identification and estimation of genetic introgression (gene flow); (*ii*) The available data for both nDNA and mtDNA diversity seemingly make introgression among many taxa of animals and plants obvious, although even in the wide *Mytilus* spp. hybrid zones, for example, introgression may be restricted or asymmetric, thus holding at least the source taxon intact; (*iii*) If we admit that sexually reproducing species in marine and terrestrial realms are introgressed, as is evident for many cases, then we should recognize that the orthodox BSC, in terms of complete lack of gene flow among species, is inadequate due to the fact that many species are not yet biological species; however, sooner or later they will become biological species. This conclusion is supported by the genetic distance, increasing with taxa rank, and by the lowest diversity at intraspecies level for single mtDNA genes, complete mitogenomes, and nDNA data; (*iv*) The recent investigation of fish taxa divergence by the author, using vast BOLD data, shows that gene trees for taxa up to family level are basically monophyletic, and interspecies reticulations are rare. **Significance:** All four listed outcomes have a general impact on the paradigms of Evolutionary Genetics, on iBOL science policy, and on the practice of species delimitation in particular. Evidently, the most common successful delimiting of species, based on barcoding technique, is possible due to the prevailing species origin throughout the geographic speciation mode.

Barcoding Slovakia as a tool for nature conservation and protection

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In 2015, the Slovak National Museum-Natural History Museum in Bratislava, Slovakia, obtained financial support of 1.7 million EUR from the EU Commission Operational Program of Research and Development and co-financed with the European Fund for Regional Development (EFRD) to build a DNA laboratory. The laboratory will serve as the central point for the barcoding of Slovak flora and fauna in 2016–2023. This laboratory has been built, and the project of barcoding Slovakia has been launched. The main research objectives by 2023 are the gathering, identifying, and sequencing of selected species of bacteria, fungi, plants, and animals from Slovakia. The aim of the research is to sequence at least 1000 species. Voucher specimens will be obtained by field research from different areas of Slovakia, from soil samples for environmental research and from collections of SNM-NHM. Activities aimed towards the conservation and protection of threatened species and habitats has already started. Of the most important are (*i*) building a barcode library of native orchid species and populations, threatened by illegal harvesting for commercial purposes; (*ii*) barcoding of reptiles and amphibians, threatened native species on one side and invasive ones on the other; (*iii*) environmental sampling on mine wastes and contaminated soils, for further analysis; and (*iv*) fungal taxonomy as a tool for better understanding interspecific relations within the habitats.

Genomic correlates of haplodiploidy: from barcodes to nuclear genomes

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Background: Variation in breeding systems impacts genome evolution. This is due to its effects on recombination rate, effective population size (N_e), and the efficacy of selection. Haplodiploidy (diploid females and haploid males) has been studied very little from the perspective of genome evolution, which is surprising given the fact that this breeding system has arisen in many arthropod lineages and is often associated with high species diversity. The aim of this study is to compare GC content and the rates of protein evolution via dN/dS ratios between diploid (DP) and haplodiploid (HDP) insect taxa, employing three extensive datasets: 76 662 COI barcodes, 13 protein-coding genes from 790 mitochondrial genomes, and over 10 000 protein-coding genes from 55 nuclear genomes. **Results:** HDP taxa had lower GC content at the 1st and 2nd codon positions than DP taxa in all three datasets studied. The same pattern was supported by sister taxa comparisons with the exception of Phthiraptera (HDP) versus Psocoptera (DP). An opposite pattern was observed in dN/dS ratios: HDP taxa had significantly higher ratios than DP taxa. **Significance:** This study reveals an interesting genomic difference between DP and HDP insects

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and raises questions on the processes generating it. As HDP taxa are amazingly diverse with regard to both species counts and life histories, the present results can help to understand the factors behind their success. Furthermore, because genome architecture has a considerable impact on evolutionary processes and little is still known about it, especially among non-model organisms, the results linking different life history traits to genomic changes are of crucial importance.

A DNA barcoding reference database for priority southern African snakes

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Background: Anthropogenic impacts on the environment have led to drastic effects on community assemblies and the diversity of species. Given their ectothermic physiological characteristics and specific niche requirements, several southern African snakes are particularly vulnerable to these effects. There is also a need for monitoring the legal trade of snake species to ensure that only non-regulated species are collected from the wild. The aim of this study was to set up a DNA barcode reference database of priority species that are currently listed as threatened or endangered on CITES appendices and IUCN Red lists and also included in permit requests for the pet trade. Look-alike species, whose specimens have similar features and can be confused with priority species that are illegal to trade for conservation reasons, were also included. **Results:** Twenty species of snakes from the families Boidae, Colubridae, Elapidae, Lamprophiidae, and Viperidae were analysed using the COI barcoding gene. A minimum of five samples per species were collected from zoos, reserves, and museum collections. The COI genetic diversity estimates revealed high levels of sequence divergence between families (17%–26%) and among species within these groups (0.2%–25%). There was also evidence of cryptic speciation and geographic variation among species from major taxonomic groups such as the vipers and colubrids with some species having more than one Barcode Index Number (BIN). **Significance:** This database indicated that DNA barcoding will be useful for snake species identification in southern Africa. The observed diversity levels further indicate that some snake families may be in need of taxonomic revaluation as there were very high levels of intraspecific variation. This DNA barcoding information will improve our current understanding of snake communities and promote more accurate prediction of future impacts of global change.

DNA barcode sequencing of *Saccharum* and wild relatives to determine level of relatedness

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Background: Genetically modified (GM) sugarcane is set to provide new opportunities to increase yield and grow the global competitiveness of the South African sugar industry. An assessment of the environmental impact is required prior to the release of such crops, especially with respect to predicting gene flow between GM crops and related wild species. The establishment of taxon relatedness is therefore important, and the ability of a short fragment of DNA sequence to discriminate between closely related species was explored for phylogenetic analysis. The aim of this study was to sequence the barcode fragment of the internal transcribed spacer (ITS) regions of the 5.8S ribosomal gene as well as that of the two chloroplast genes, ribulose-bisphosphate carboxylase (*rbcL*) and maturase K (*matK*), to determine relatedness between *Saccharum* and its wild relatives. It is generally agreed that a plant barcode will combine more than one locus, a phylogenetically conservative coding locus (*rbcL*) with a rapidly evolving region (*matK*). Genomic DNA was extracted, PCR amplified, and fragments were sequenced (Applied Biosystems 3500 Genetic Analyser using a BigDye Terminator V3.1 Sequencing kit). **Results:** While modern sugarcane cultivars are thought to have arisen from an interspecific hybridization between *Saccharum officinarum* and *S. spontaneum*, the genus *Miscanthus* seems to be the most closely related to this germplasm. Further results from this study are expected to demonstrate sufficient sequence variation to allow phylogeny structuring through maximum-parsimony, neighbour-joining, and maximum likelihood tree analyses. **Significance:** The combination of all three loci is expected to improve resolution of the phylogenetic analyses, enabling the use of these DNA barcodes for consideration as preferential choices for relatedness studies of the tribe Andropogoneae.

Use of DNA technology in combating illegal trade and promoting conservation and sustainable use of plants in Kenya and Tanzania

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Background: Trafficking, poaching, and illegal trade in wildlife affect Kenya and Tanzania. Plant traffickers poach, modify, and ship them in forms not easily identifiable using morphological methods. This illegal trade, sustained by a complex global black market worth billions of dollars annually, is the largest threat to the survival of wild populations of useful plants. The rich natural diversity of plants, valued and exploited as herbal remedies, beauty products, ornamentals, timber, etc., has potential to generate new streams of incomes if sustainably exploited. The project aims to generate DNA barcodes and create a reference library, for identification and verification of plant product samples; a database of plants in trade; promote sustainable use for ecosystem security and economic development and enable natural resources and CITES Management authorities in Kenya and Tanzania to investigate and prosecute trade-mediated wildlife crime. **Results:** Traded plants and derivatives were documented in markets in Kenya and Tanzania, using questionnaires, observation, and sampling confiscated and suspected contraband materials, at ports of entry/exit and Phytosanitary offices. The project targets to add 2000 "barcodes" in GenBank with over 500 samples undergoing analysis. Herbal medicine contributes the highest diversity of wild-sourced plants in trade. Lowland dry forests in southwestern Kenya and southeastern Tanzania are home to most of the region's wild flora and the primary area of collection, and occasionally from Uganda and the East Africa region. Traders use local tribal names, making it difficult to record and regulate the trade. **Significance:** DNA technology in plant exhibit verification and identification for wildlife crime investigation is unconventional in this region. Two expert evidence reports have been submitted for ongoing court cases using the DNA reference library, and four others are being analysed. To make the technology widely acceptable, Tanzania collaborators have been trained in DNA barcoding and are undertaking a proficiency test.

Comparisons of the mycobiome of five underutilized crop species in an intercropping system using next-generation sequencing

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Background: In order to achieve a desirable ecological and sustainable agriculture, a thorough understanding of the plant–soil myco-

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biome of a crop is imperative. Next-generation sequencing (NGS) enables rapid analysis and comparisons of the composition and diversity of microbial communities in any habitat. This was the goal of this study for soil and plant niches of five crops in an intercropping plot. These included bulk and rhizosphere soils, internal plant tissues, and seeds of Bambara groundnut, cowpea, drybean, soybean, and sorghum, including a fallow treatment. The internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA was used. **Result**: The highest fungal diversity was recorded by the Ascomycetes followed by the Basidiomycetes and Zygomycetes. Principal coordinate analysis indicated degrees in overlap and differences between the mycobiomes of the various crops, and between the various niches. The legumes tended to cluster together while sorghum formed a group on its own. Clear clustering was also observed between soil and plant-associated fungi, and rhizosphere and bulk soils. The most prominent genera in all the samples investigated were *Phoma*, *Fusarium*, *Cladosporium*, and *Cryptococcus*. Pathogen genera detected were *Alternaria*, *Epicoccum*, *Colletotrichum*, *Myrotherium*, *Thecaphora*, *Ustilago*, and *Sporisorium*. Genera such as *Emericella* was specific just in cowpea, *Chaetomium* specific to soybean, and *Sporisorium* and *Ustilago* found in sorghum only. Drybeans had the highest percentage of fungal abundance (17.2%), followed by soybean (13.8), bambara (12.4%), cowpea (8.8%) and sorghum (7.1%). The above-ground samples were dominated by *Cladosporium* and *Phoma*, while the below-ground parts were dominated by *Fusarium*. *Significance*: Through NGS technology we effectively captured the composition of the mycobiome in the different crop species and substrates in a complex system. Continued monitoring using this approach will enable us to study the various plant–soil–mycobiome interactions to increase crop yield and health.

DNA barcodes reveal new species of leaf-mining moths from Siberia and the Russian Far East forests and illuminate the invasion process of some species

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Background: DNA barcodes are an excellent tool for rapid biodiversity assessments of poorly known areas of the world. The insect fauna of the vast forests of Siberia and the Russian Far East is still poorly known, with many species still to be discovered and described. With rapid climate change some of those forest insects are expected to expand their distribution ranges and become serious pests. Among forest insects, leaf miners represent an important group, with many important pests. Here, we develop a DNA barcoding reference library of leaf-mining moths of Siberia and the Russian Far East. **Results:** So far we have obtained 580 DNA barcodes of 65 leaf-mining moth species from nine different families (Bucculatricidae, Eriocraniidae, Elachistidae, Gracillariidae, Incurvariidae, Lyonetiidae, Nepticulidae, Tischeriidae, and Yponomeutidae) developing on 72 woody plants from 10 different families (Adoxaceae, Betulaceae, Cornaceae, Fagaceae, Fabaceae, Malvaceae, Oleaceae, Rosaceae, Salicaceae, and Ulmaceae). The DNA barcoding data revealed the presence of several central European gracillariid species in Siberia and the Russian Far East for the first time. In addition, we discovered five new candidate species that are in the process of being described. Barcode data revealed an unusually high genetic diversity of the invasive lime leaf miner in the invaded region (Europe) compared to the native region (eastern Asia), suggesting a scenario of multiple introductions. **Significance:** This is the first study to assess the true diversity of leaf-mining moths from Siberia and the Russian Far East using DNA barcodes.

Using eDNA from soil and Malaise traps to monitor renaturation measures in a European forest

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In the early 1970s a massive loss of biodiversity was recognized, and since then National Parks (NPs) have gained growing importance as it is their major task to restore and sustain the native fauna and flora. To meet these demands, several areas within the young Eifel NP in western Germany are currently undergoing comprehensive renaturation activities. To manage these measures effectively, the ecological restoration progress needs to be monitored regularly. Further than just basing a study on a very limited number of indicator species, biomonitoring can be achieved by using all the available biodiversity as a proxy for the degree of renaturation. By using environmental DNA (eDNA) together with next-generation sequencing (NGS) platforms, it is now possible to access unprecedented levels of biodiversity, and use this wealth of information to comprehensively evaluate changes in species composition in a timely and cost-efficient manner. This metabarcoding study targeted invertebrate diversity throughout one year at 14 sampling sites located in the Eifel NP. These sampling sites differed in management form and represented replicates along a transitional gradient of spruce to beech forest including underplantation measures. In addition to direct extraction of DNA from the preservative ethanol in Malaise traps, eDNA was also extracted from soil samples taken at the same sampling sites. In order to allow for a better taxonomic resolution and wider taxonomic coverage, the mitochondrial COI barcode region together with nuclear 18S rRNA genes were PCR amplified. This study highlights the advantages of metabarcoding for natural resource management and conservation purposes. Furthermore, it will provide insights into community composition and ecological patterns triggered by human-controlled renaturation activities.

Building the DNA barcode library of Holarctic Mycetophilidae (Diptera)

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The some 30 000 DNA barcoded fungus gnats (Diptera, Mycetophilidae) of the Holarctic Region, forming 1800 Barcode Index Numbers (BINs), are analysed with respect to sampling efforts, fauna composition, and species boundaries between the Nearctic (NA, largely Canada) and the Palaearctic (PA, largely Scandinavia) continents. Passive mass sampling in NA (86% of barcodes) have yielded 1210 BINs (67%), while a more targeted sampling in PA (14% of barcodes) have yielded 760 BINs (42%). Geotactic collecting methods proved more efficient (720 BINs with average of 9 sequences/BIN) than did heliotactic collecting methods (1190 BINs with average of 20 sequences/BIN). The species composition richly represents all major subfamilies and most genera on both continents, the tribe Exechiini being extraordinarily well represented with 624 (35%) of the BINs. Altogether 160 BINs (21% of PA, 13% of NA, 9% of all) are shared between the continents. Among the Scandinavian barcodes, where the taxonomic precision based on morphology is relatively high, a very good match between morphology and BINs is documented, leaving only a tiny fraction of discordant BINs and a somewhat larger proportion of potential splits yet to be analysed. The taxonomic precision of the Canadian barcodes has been greatly improved through online inspection of voucher images and ID-trees. This enabled genus placement of nearly all BINs, but lots of efforts remain to obtain species-level precision. A continuous curating and maintenance engagement is essential in order to develop and refine the DNA barcode library as a high-quality reference for next-generation sequencing studies.

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Metabarcoding with environmental DNA to identify wildlife species potentially attracted to uranium mine containment ponds as a water source in the arid southwest US

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Background: Development of new uranium mines in the Grand Canyon watershed in northern Arizona was restricted in 2009 by the federal government until studies assessing the potential impacts of radionuclide and heavy metal contamination could be completed. Water containment ponds at mines are designed to receive all surface run-off and contain elevated chemical concentrations. The ponds are also a constant water source in an arid region and could result in contaminant exposure to local food webs. To understand the heavy metal exposure pathways, we conducted environmental DNA (eDNA) metabarcoding in parallel with traditional biodiversity surveys via small mammal trapping and acoustic monitoring to identify wildlife using these water sources. With samples from surface water near active mines and mine containment ponds we employed a metabarcoding approach with 12S and 16S rRNA gene markers. **Results:** Using the 12S markers we recovered large numbers of sequence reads from taxa expected to be in the area and from less common or hard to observe taxa such as the Mexican free-tailed bat and the tiger salamander. Detection of the tiger salamander is of note because this species was not observed by the traditional biological survey techniques used. Due to low phylogenetic resolution of the 12S marker, most taxa were not identified down to species level. Using our 16S markers, we expect to improve our taxonomic resolution. We will compare our metabarcoding survey results with those from the traditional survey methods and also look at seasonal changes in species occurrence. **Significance:** As eDNA is quickly becoming a popular tool for wildlife surveys; we will discuss the advantages and limitations of this technique based on our experience. Ultimately this tool will enable us to better understand the overall biodiversity of the area and aid risk assessment of resuming new mining activities.

Monitoring marine biodiversity in the North-East Atlantic using DNA barcoding, proteome fingerprinting, and environmental DNA analysis

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During the last years, the metazoan diversity of the North Sea, located between the British Isles and the mainland of northwestern Europe, was extensively studied using different molecular methods. A DNA barcode reference library was established for a broad range of animal taxa including economically important groups like fish, mollusks, and crustaceans. Besides DNA barcoding, more cost and time efficient methods were tested in order to provide accurate low-cost species identification. In this context, the most promising method was the analysis of proteome fingerprints by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Based on species and individuals, a priori identified to species level using morphological diagnostic characters and DNA barcodes, proteome fingerprints were generated using MALDI-TOF MS. Pilot studies and preliminary results for different metazoan taxa including different life-history stages demonstrated species-specific proteome fingerprints and thus a promising supplementary or alternative successful species discrimination and identification method. After the establishment of a comprehensive proteomic library, we aim at identifying species within minutes and for few cents of material costs only. A possible application for proteome fingerprinting is the rapid identification of environmental samples in the perspective of monitoring (i.e., zooplankton, fish eggs/larvae surveys). In contrast, methods based on non-invasive sampling will become more and more important for monitoring purposes in the future. Here, we obtained environmental DNA from water samples from different locations in the German Bight. The DNA was sequenced using newly designed COI mini-barcode primers and an Illumina next-generation sequencing platform. Obtained sequence data was compared with the COI DNA barcode reference library, in order to check for species coverage.

In sickness and in health: microbiota dynamics of the solitary bee species *Osmia bicornis* (Linnaeus, 1758)

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Background: The red mason bee (*Osmia bicornis*) is a solitary bee species well known for its services as a generalist pollinator. In this study, *O. bicornis* natural nest microbiota underwent thorough investigation to gain insights into the microbial contribution to larval health. A DNA metabarcoding approach using Illumina next-generation sequencing of 16S ribosomal DNA was applied on 99 nest chambers originating from differently structured landscapes. **Results:** Sequencing of a total of 291 larvae, pollen, and nesting material samples resulted in 6630 bacterial taxonomic units, 79.6% of which were classifiable at family level, while 57.8% were also assignable to genus level. We identified several microbial taxa as associates or pathogens according to their reported function, related their presence to ecological factors, and also compared them to those of other co-existent solitary bee species. Diversity and differences between microbiota were assessed using broadly applied alpha and beta diversity indices, while random forest classification was used to distinguish microbial taxa according to host. In the direction of characterizing different taxa as commensal or mutualistic in terms of nutrition and pathogen defense, we designed functional bioassays to check culture-isolated bacterial strains for possible fermentative and antimicrobial abilities. **Significance:** Temperature shifts and landscape degradation have been set in the center of bee health research; there is still though a high level of larval mortality that remains unexplained. Studies on honey bee guts have revealed microbial agents that are assumed to adopt key functions in the hive. Microbial assessments of solitary bees, not rejoicing in the benefits of a hive system, are currently lacking. To further study microbial contributions to solitary bee development and health, our future research will include more species, focus on bioassays and investigation of genomes of microbes possibly undertaking services such as bioconversion of pollen and organism immunity.

Barcoding of marine invertebrates from Norway through NorBOL

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The Norwegian Barcode of Life (NorBOL) project endeavors to assemble and include validated barcodes of 20 000 eukaryote species found in Norway into the Barcode of Life Data System (BOLD) by the end of 2018. The University Museum of Bergen has mainly focused on barcoding marine invertebrates. Obtaining species-identified material for DNA sequencing is logistically challenging because marine sampling is expensive and taxonomic expertise is scarce for many groups. We collaborate closely with ongoing surveys (e.g., MAREANO), which supply fresh ethanol-fixed material from a wide range of localities. Through the Museum's own efforts and those of other projects funded by the Norwegian Taxonomy Initiative, we have so far submitted over 7000 marine invertebrates from close to 3000 species to Sanger sequencing of the COX1 barcode region at the Canadian Centre for DNA Barcoding (CCDB). Our experience suggests that the current high-throughput methodology is not able to consistently produce good quality sequences over the whole spectrum of species groups. On

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average, we are getting barcodes for 65% of the specimens, and for 75% of the species submitted. This shows that we miss a considerable part of the known species diversity with the standard COX1 barcoding procedures. We suggest that this may sometimes be compensated with additional sequencing of 16S fragments. We observe that BOLD, with incorporated GenBank data, has a considerable proportion of taxonomic discordances. Many of these issues will require integrated studies by taxonomic specialists. Cases of apparently over-split species are indicated by identical sequences. Much more frequently, we come across morphologically defined species with genetic divergence, sometimes as high as 30%. DNA barcoding has therefore also become valuable for higher-resolution data on spatial distributions of significant evolutionary units, and for initial discovery of new species.

Assessing species diversity in marine bristle worms (Annelida, Polychaeta): integrating barcoding with traditional morphology-based taxonomy

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Background: The marine fauna of the Nordic countries has been regarded as well known, with a history of species descriptions dating back to Linnaeus in the 18th century. Presently, 725 named species of marine bristle worms are known from Norwegian waters. Nevertheless, a number of species appear to represent complexes of confounded species, and recent studies have documented the presence of cryptic species. As a part of the Norwegian Barcode of Life (NorBOL), and with support from the Norwegian Taxonomy Initiative, a large-scale effort aiming at genetically characterising the polychaete fauna is in progress. Norwegian waters span a wide range of habitats with variable topography from fjords and coastal waters to deep shelf areas and abyssal waters. **Results:** At present, 3000 specimens of 400 morpho-species have been submitted, and have so far yielded 1600 barcodes that group into 500 Barcode Index Numbers (BINs). The sequencing success rate for species varied from 40% to 100% using the standard procedures, though certain genera and species have proven particularly difficult. Even with an average success rate just over 50%, the analyses have revealed presently unknown diversity in all polychaete families represented. **Significance:** Barcoding is a powerful technique for species discovery and discrimination, providing vast amounts of biodiversity information. Validating sequences as barcodes to species requires particular knowledge of the history, practice, and current state of the taxonomy obtained with traditional methods. In cases when nominal species cluster to multiple BINs, application of a broader set of species delimitation techniques is required, and it may be necessary to obtain genetic data from type localities to correctly associate barcodes with valid taxa names. Our results are promising for building a Norwegian DNA library, although some families are challenging. It appears that the polychaete diversity in Norwegian waters is at least 30% higher than presently known.

Barcoding subterranean beetles: a tool for taxonomic identification and evolutionary biology

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Background: An authentic classification of species is a pre-requisite for research in ecology and biodiversity. Lack of taxonomic understanding has been a major impediment to the study and management of scarabaeid beetles. Proper identification of the species and knowledge of their bio ecology is essential for developing environmentally compatible integrated pest management strategies. Identification of subterranean scarabaeid species is a challenging task due to variable morphological differences among species and delineation among the immature forms, the grubs and adults. The limitation of conventional morphological taxonomy warrants simpler methods of identification. DNA barcoding facilitates prompt identification of the pest utilizing fragmentary body parts. **Results:** In this study, barcodes were generated to identify the scarabaeid beetles from various geographical locations in South India, based on the mitochondrial cytochrome *c* oxidase I (COI) gene. Genomic DNA of 23 scarabeaid beetles were characterized, and a total of 19 barcodes were generated with Barcode Index Numbers (BINs) in the Barcode of Life Data System (BOLD). Evolutionary relationships and, divergences were assessed using MEGA programme and neighbour-joining (NJ) methods; nucleotide composition, genetic variations, and sequences similarities were calculated. **Significance:** The implications of the information generated for species delineation of scarabaeid beetles and increased accuracy in their management is discussed.

Assessment of the aquatic community ecology of Culicidae in Kruger National Park using environmental DNA

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Background: In recent decades we have witnessed the re-emergence of mosquitoes and mosquito-related diseases. Accurate descriptions of mosquito populations and community ecology remain limited, largely due to logistical challenges in identifying and quantifying mosquito species. Particularly, knowledge on the reproduction sites is scarce, with regards to the different preferences for different mosquito species. Mosquitoes require an aquatic environment to lay eggs, for larvae to hatch, instars to change, and adults to emerge. Understanding the community composition and abundance, including disease vectoring species, may provide the tools for better management, but this requires much better in-depth knowledge of the ecological requirements of the main disease-vectoring species. Here, we aim to develop and validate an environmental DNA (eDNA) survey method to investigate mosquito community composition and species abundance. **Results:** To test this method, we conducted a field study inside the Kruger National Park (KNP) and in the fringing rural communities. At each site, adult mosquitoes and mosquito predators were caught with terrestrial traps and aquatic eDNA water samples were taken. To improve the DNA barcoding database, all different species collected were barcoded. Furthermore, to calculate degradation of DNA under standard conditions, we carried out a field mesocosm experiment with different densities of a single species that was dominant across our field sites. We expect that our eDNA approach will show similar results in comparison with the terrestrial traps. Our preliminary results show a difference in mosquito and mosquito predator community composition and abundance between inside and outside KNP. Inside the park the communities are diverse, and outside the park communities are often dominated by a single species. **Significance:** Using an eDNA approach, we hope to facilitate more in-depth research to improve knowledge on the ecology of mosquitoes, in particular the disease-vectoring species, their predators, and the relationships with abiotic variables.

DNA barcoding of Austrian molluscs: challenges and success

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Background: In the course of the Austrian Barcode of Life (ABOL) initiative, molluscs were chosen for several reasons for a 3-year pilot study.

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There are important indicator species, suitable for evaluation of habitat quality. This leads to a high risk of extinction for many molluscs: about 35% of the snail and 37% of the mussel species are endangered. Also, the number of endemics is quite impressive (19.3%). Approximately 30% of the 400 native species are divided into subspecies. Genetic investigations in land pulmonates showed extremely high intraspecific diversity. Hence, there is no standard value for genetic distances, which marks taxonomic delimitations. Due to the overlap of intra- and interspecific variation, often no barcoding gap can be found. Previously collected data and experiences from past and running projects on snail species in Austria provide excellent pre-conditions for successful DNA barcoding. **Results:** Up to now, ~250 Austrian mollusc species are available. This includes material collected and preserved for DNA analyses during concerted field trips to different parts of Austria, but also older material from the collections of the Natural History Museum Vienna and the Biology Centre of Linz. DNA from museum material is often fragmented and of low concentration; therefore, it is only chosen when no other material is available (~35 species). To date, from 185 different species, 548 DNA barcodes with all relevant metadata were established. From our results we find cryptic species, but also different morphologically described species that cluster in one Barcode Index Number (BIN). **Significance:** This project provides DNA barcodes for a challenging group of molluscs. Data are sampled at a relatively small geographic scale, which is essential for taxa with low dispersal capacity. The DNA barcodes will facilitate determination, which is often difficult and vague in molluscs; hence, DNA barcodes are highly useful in performing identifications for nature conservation.

Barcoding fauna of India: an initiative by Zoological Survey of India

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Background: The Zoological Survey of India (ZSI), since its inception in 1916, has been maintaining several type materials in its National Zoological Collections at headquarters in Kolkata as well as in different regional museums. ZSI is the premier institute on faunal research containing over 5 million specimens, including more than 17 000 type specimens from Protozoa to Mammalia, from India and more than 60 other countries. Integration of molecular data with morphology is one of the major mandates for ZSI. To date, over 2000 DNA barcodes for a number of species have been contributed to GenBank and the Barcode of Life Data System (BOLD), including groups such as economically important insects, indicator species of Lepidoptera, freshwater fishes, reptiles, and wildlife seizures. **Results:** The study of barcode data from various groups resolved several problems of their identification, taxonomy, and distribution. To name a few, in lower groups of animal, we detected alien insect pest species (*Thrips parvispinus*), detected cryptic diversity in the insect order Thysanoptera (for species complexes, *Frankliniella schultzei* and *Thrips palmi*), and detected host-specific diversity in Hemiptera (*Helopeltis theivora*). In Araneae, we recorded the species of the genera *Neriene* and *Psechrus* for the first time from India. In higher animals, we for the first time recorded the distribution of a fish of the subfamily Gobionellinae, order Perciformes, from northeast India. We further detected three non-native species of turtles and tortoises from northeast India (*Chitra chitra*, *Cyclemys fusca*, and *Amyda ornata*) along with the detection of *Nilssonia nigricans* from wild habitat, though it has been categorized as "Extinct in Wild" in the IUCN Red List. **Significance:** DNA barcoding has a long way to go for covering all the extant species to achieve its real advantage in biodiversity research and conservation. Our initial success from the Indian region motivates future collaborative endeavours for barcoding and generating mitogenomes from the archival specimens.

Barcoding and phylogeography in clownfishes (Perciformes: Pomacentridae) of the Indian Coast

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Background: Coral reef fishes are distinguished primarily by their color patterns. However, closely related species show only slight variations in their color patterns, not to mention colour variations at different life history stages. Molecular taxonomy studies have shown that color variations are not always sufficient indicators of genetic isolation and species boundaries. Barcoding of 13 clownfish species of the Indian waters was carried out to infer the phylogenetic relationships among them by analyzing mitochondrial genes cytochrome oxidase 1 (COI) and cytochrome *b* sequences. The study also scrutinized species boundaries between four closely related species of the subgenus *Phalerebus*, three species of the subgenus *Amphiprion*, and two species of the subgenus *Paramphiprion* by analyzing the mitochondrial control region (CR). In addition, phylogeographic structure of *A. clarkii* was calculated in terms of geographic isolation by phylogenetic analysis of mitochondrial control region and cytochrome *b* sequences. **Results:** The genetic distances between the species of subgenus *Phalerebus* were 0.165–0.233 in the control region and 0.021–0.065 in cytochrome *b*; and the genetic distance between the species of subgenus *Amphiprion* was 0.122–0.171 in the control region and 0.038–2.308 in cytochrome *b*. Species of the subgenus *Paramphiprion* had a genetic distance of 0.016 in the control region and 2.185 in cytochrome *b*. *A. clarkii* collected from four regions have genetic distances of 0.019–0.06 (control region) and 0–0.025 (cytochrome *b*). With increasing application of DNA barcoding, many previously unrecognized fish species will be revealed through the discovery of deep divergence of COI sequences within currently recognized species. **Significance:** Phylogeography of *A. clarkii* revealed that the variations were present within the species with respect to the features of their geographical areas. Results of this study disclose that the morphologically similar species of each subgenus of clownfishes are closely related, as supported by the molecular phylogeny data.

Barcoding of reef fishes of India

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Background: Molecular techniques based on the analysis of short, standardized gene sequences for rapid, accurate, and automatable species identifications have been adopted as a global bio-identification system by systematic ichthyologists and fishery biologists. In most animals, a fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) has been used as the target sequence. The present barcoding of reef fishes inhabiting the coral reefs in Indian waters (Laccadive and Arabian Seas) is a pioneering venture. **Results:** In the current study, 68 species of fish belonging to 16 families were barcoded. Some species were represented by multiple specimens. Following amplification and sequencing, a total of 91 sequences were generated. All related specimens formed cohesive units and were separated from each other in the maximum likelihood tree, allowing their unambiguous identification in concurrence with the taxonomic status of the species. BLAST analysis of each species showed 99% similarity with sequences of similar species available in GenBank, confirming the identity of the specimens. In addition to barcode-based species identification, phylogenetic relationships among the species were also studied. **Significance:** More importance should be given to launch global comprehensive reference DNA barcoding libraries. Traditional taxonomists will also play an important role in preparation of such databases by incorporating morphology with molecular taxonomy. Once a COI barcode database has been established for fishes, the identification process can be made easy and highly precise. Above all, identification will be possible from fish eggs, larvae, or carcass fragments. This will be an invaluable tool for researchers, fishery ecologists, and aquarists.

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Molecular characterisation and plasmid profiling on bacteria isolates from Naraguta dumpsite, Jos, Plateau State

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Background: Environmental contamination is a global issue due to increased industrialization and anthropogenic activities. The aim of this study was to identify bacteria with contaminant-degrading abilities. Effluent from the dump site was collected in a new sterile container and cultured on nutrient agar. The pure colonies were further transferred to nutrient broth. These isolates were stained using Gram staining techniques. The isolates then underwent biochemical analysis including oxidase, urease, catalase, indole, and citrate tests. The extraction of DNA from the pure isolates was carried out using ZymoBeadTM DNA extraction Kit. Polymerase chain reaction (PCR) was carried out to amplify 16S rRNA and gyrase B gene. Agarose gel electrophoresis was carried out to visualise the output of the PCR reaction using 1% agarose. Unpurified PCR products were sent for sequencing. The sequences generated were further analysed using bioinformatics tools. Plasmid isolation was carried out on the isolates using Monarch® Plasmid DNA Miniprep Kit, and it was then run on gel electrophoresis to visualise the output. **Results:** Plasmid profiling showed that the isolates had one plasmid each of size 4 kb. The bioinformatics results showed the organisms *Pseudomonas aeriginosa* strain HOB1 (92%), *Pseudomonas* sp. CN2 (92%), *Pseudomonas aeriginosa* strain E1 (91%), *Pseudomonas aeriginosa* strain KRF 102 (92%), uncultured bacterium (91%), sequence and BTEX degrading bacteria (68%), *Stenotrophomonas maltophilia* strain M2 (72%), *Stenotrophomonas maltophilia* strain ISSDS (66%), *Stenotrophomonas* sp. 2012A (68%), and uncultured bacterium clone D25-814 (66%). **Significance:** Plasmid profiling showed these isolates contain one plasmid each with a size of 4 kb and contain resistance for chloramphenicol and ampicillin. The percentage similarity between the isolates and the bacteria in the database shows that there is further need for an intensive study on these isolates to ascertain whether these isolates are the same as any of the bacteria in the database or new strains.

The first DNA barcode reference library for mosses: *rbcL* and *trnL-F* for 775 species of Bryophyta from Canada

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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 a replacement marker. The plastid-encoded intergenic spacer *trnL-F*, a widely adopted marker for mosses due to its universality and high variability, is a promising second DNA barcode for mosses. **Results:** We tested sequence recovery and species resolution with *rbcL* and *trnL-F* for 775 Canadian moss species, about three quarters of the Canadian flora. Samples of nearly 2000 specimens from the herbarium at the Canadian Museum of Nature were analyzed at the Centre for Biodiversity Genomics. Standard protocols recovered *rbcL* from 94% of the species and *trnL-F* from 98%. A maximum likelihood phylogeny using these markers suggested the polyphyly of some families (e.g., Rhabdowesiaceae, Dicranaceae). The complementary resolution power for *rbcL* and *trnLF*, although variable among orders (Sphagnales 32%, Splachnales 100%), allowed the discrimination of about 60% of the species examined and all 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 re-identified after reexaminations provoked by the barcode results. **Significance:** DNA barcoding will provide critical insight into the alpha and beta taxonomy of Canadian mosses and improve the quality of identifications on 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.

Finding the weeds through the pond: eDNA reveals underestimated diversity of pondweed species (Potamogetonaceae) using water samples

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Background: The detection of eDNA with high-throughput sequencing has rapidly emerged as a method to detect organisms from environmental samples. Environmental DNA (eDNA) studies of aquatic biomes have focused on surveillance of animal species with less emphasis on plants. The pondweeds are one of the most important components of macrophytic plant communities in freshwater ecosystems, providing food and shelter for fishes, birds, macroinvertebrates, and plankton. Identification of pondweeds is especially problematic because aquatic habitats are often less accessible than terrestrial ones. Additionally, microscopic characters make their authentication challenging, particularly for non-specialists who assist with field work. We used water samples to track pondweed diversity at the Grand River at the Charitable Research Reserve (RARE). **Results:** We developed an eDNA protocol to detect pondweeds in water samples using specific DNA markers with species-level discriminatory power (*atpB-rbcL* and ITS2). Short fragments (168–215 base pairs) were successfully amplified using primers targeting genera from the pondweed family (*Potamogeton*, *Stuckenia*, and *Zannichellia*). We identified the presence of seven species at three sites in RARE. In addition to two species previously collected at the site (*Stuckenia pectinata* and *Potamogeton crispus*), we detected five species new to the reserve (*Stuckenia vaginata*, *Potamogeton friesii*, *Potamogeton alpinus*, *Potamogeton subsibiricus*, and *Zannichellia palustris*). Our data reveal that diversity of pondweeds is possibly higher than predicted by traditional morphological surveys. **Significance:** Our study uses a targeted approach to track the species composition of pondweeds in freshwater ecosystems. A detected combination of species of pondweeds can work as a "fingerprint", or indicator for a particular environment, biological community, or water quality. This result suggests that eDNA can be an efficient tool for monitoring plant diversity in aquatic habitats.

eDNA metabarcoding as a new surveillance tool for coastal Arctic biodiversity

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Background: Because significant global changes are currently underway in the Arctic, creating a large-scale standardized biodiversity database for Arctic marine biodiversity is particularly pressing. Marine monitoring typically requires large and expensive sampling tools, multiple experts and may sometimes have negative impacts on the habitat,

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limiting the capacity for detecting biodiversity changes in such a large ecosystem. Environmental DNA (eDNA) metabarcoding, which involves less challenging and intrusive field methods, could be a revolutionary tool for overcoming the lack of extensive biodiversity data. However, eDNA from metazoans has mainly been used in freshwater systems, and its efficacy for detecting biodiversity shift needs to be evaluated in other ecosystems. Our goal was to evaluate the potential of eDNA metabarcoding in assisting with sustainable development in coastal areas of the Canadian Arctic by generating new biodiversity monitoring tools for the marine ecosystem. The eDNA was extracted from ~80 water samples collected in two Arctic Canadian ports (Churchill and Iqaluit) and amplified using two COI primer pairs. We (*i*) evaluated the efficacy of eDNA metabarcoding to assess coastal biodiversity in Arctic commercial ports, (*ii*) contrasted community structure among sampling locations (i.e., water column depths and tide pools), and (*iii*) evaluated seasonal variability. **Results:** We successfully used eDNA metabarcoding of water samples to monitor coastal metazoan species in the Arctic. We showed that eDNA is spatially and temporally heterogeneous within ports and that the efficiency of the eDNA monitoring surveillance is improved when sampling under-ice cover. **Significance:** By allowing rapid sample collection by inexperienced or novice individuals, reducing the cost associated with data collection/shipping and reducing manipulation of organisms, the analysis of eDNA from water samples could be a revolutionary tool to increase the power of detection, spatial coverage, and frequency of sampling, thus improving detection of biodiversity shifts in large coastal Arctic ecosystems.

DNA barcodes and the comparative phylogeography of North American Lepidoptera

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Background: With the surge in barcode data, there is a growing gap between the availability of sequences and the effort directed toward their analysis. BOLD, the Barcode of Life Data System, now holds over 5.3 million barcode records, deriving from over 500 000 species, albeit many still unnamed. More than half of these records are insects, and over a million records are from the order Lepidoptera. While these barcodes have been used extensively in studies of biodiversity, species identification, and phylogenetic relationships, little effort has been directed toward the examination of intraspecific diversity. Fewer studies have examined how phylogeographic patterns vary among species within and across families, habitats, and life history strategies. **Results:** This study examines patterns of intraspecific population genetic structure in several lepidopteran superfamilies, with a focus on the Bombycoidea. DNA barcodes were retrieved for 1856 specimens from 54 bombycoid species representing three families and seven subfamilies. The number of variable sites in COI ranged from 0 in four species to over 50 in three species. The resulting population genetic structure varied depending on taxonomy, species distribution, and life history traits. **Significance:** Barcode data for the lepidopteran fauna of North America is now sufficient to study intraspecific variation in many species, making it possible to detect common patterns resulting from ecological and physical barriers. These records also make it possible to study how life history characteristics influence gene flow and dispersal, providing a better understanding of the presence and movement of species. In addition, DNA barcodes play a crucial role in identifying species of interest for further evolutionary study.

DNA barcoding the aquatic biodiversity of India

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Background: With its four global biodiversity hotspots (North East Region, Western Ghats, Himalayas, and Nicobar Islands), India harbours a wonderfully diverse aquatic fauna that significantly contributes to the world's biological resources. The country possesses 2868 indigenous fish species, including 877 freshwater, 113 brackish, and 1878 marine species. This total excludes ~400 exotic fish and shellfish species. In addition to finfish resources, there are 2934 species of crustaceans (2430 marine, 504 freshwater), over 5000 species of molluscs (3370 marine, 1700 freshwater), 765 echinoderms, 486 sponges, and 844 seaweeds. **Results:** These aquatic bioresources are being authenticated using DNA barcoding and allied techniques for biodiversity assessment, utilization, and management. As the leading research group in the region, we have generated barcodes of over 500 species of fish, crustaceans, and molluscs. As well, we have trained several national and international researchers in biodiversity genomics. **Significance:** Our recent work on barcoding seafood of India has revealed over 30% mislabeling in restaurants, which is of great concern. This presentation will also highlight recent initiatives within India including new project funding, national, regional, and global collaborations, human resources development, barcode data management, and the increased use of DNA barcoding by Indian regulatory and forensic agencies.

Low coverage genome sequencing of species of Saliceae: the quest for additional markers

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Background: Shared haplotypes of the chloroplast genomes between multiple species of *Salix* and *Populus* (tribe Saliceae) means that the traditional plant barcode markers *matK* and *rbcL* are unable to distinguish between species. The ribosomal, mitochondrial, and nuclear genomes can yield more informative regions. Transposable elements (TE) are highly repetitive DNA segments that can provide their own means of propagation in the genome and are thus present at high copy numbers. The retrotransposons class of TEs (named after their reliance on reverse transcriptase to multiply) are the most abundant group of TEs in plant genomes. The high copy number of these TEs means that they can be sequenced using low-coverage genome sequencing method, similar to the plastid genomes. Furthermore, the fast-evolving nature of the TEs means they can provide more insight into the Saliceae phylogeny and act as novel markers for identifying species. **Results:** In total, 63 species of Saliceae (83 individuals) were low-coverage genome sequenced: 40 Norwegian individuals (4 *Populus* and 36 *Salix*) from herbarium material and 43 Alpine individuals (4 *Populus* and 39 *Salix*) from fresh material dried in silica gel. Additionally, two outgroup species of Salicaceae were sequenced (*Casearia* and *Xylosma*). Preliminary assembly results yielded the full ribosomal and mitochondrial genomes and ~50 homologous TEs in addition to the full chloroplast genomes. Furthermore, the first assembled TEs indicate that they contain enough interspecific variation for phylogenetic analysis. **Significance:** This is the first study that uses low-coverage genome sequencing to both obtain TE data and use it to solve taxonomic issues. The methods applied in this study provide an efficient way to maximise the results that can be obtained from low-coverage shotgun sequencing efforts and sheds light on how TEs can be used as additional markers to solve complex phylogenies.

Multifaceted DNA metabarcoding for noninvasive studies of bats

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To measure important attributes of bat ecology, advanced methods are needed that minimize stress to individual bats and disturbances to

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colonies, and have low risk of spreading White-Nose Syndrome (WNS) among bat populations. We are testing the accuracy and applicability of multifaceted (or multiple data class) DNA metabarcoding (MDM) to provide species identifications, sex identifications, diet analyses, parasite analyses, and WNS detections, as well as individual genotypes or fingerprints, from sample sets comprised of numerous individual guano pellets. Using an Illumina® MiSeq platform for amplicon resequencing, we have tested MDM on guano samples obtained from multiple bat species and locales. Based on species we have tested and other published studies, we should at this point be able to use DNA from individual guano samples to (*i*) identify taxon for at least 75% of bat species in the continental United States, (*ii*) correctly determine sex for at least 50% of those bat species, (*iii*) detect the fungal agent (*Pseudogymnoascus destructans*) of WNS in nearly 100% of cases, (*iv*) effectively describe diet using several different arthropod and plant barcodes, and (*v*) more effectively characterize endoparasite diversity. Optimization of DNA microsatellite genotyping within the MDM run is on going. Interesting results to date include the observation that 16S rDNA barcode primers designed for arthropods also often provide accurate bat species identification. While the endoparasite barcode data provided by MDM was highly informative, we were unable to detect ectoparasites. Not unexpectedly, we have observed some cases of either DNA cross-contamination among samples or "index-swapping" that would result in inaccurate MDM results. So, while MDM shows significant promise as a tool for noninvasive studies of bats, laboratory and analytical protocols that maximize data quality and minimize risks of false positives will be critical components of any study.

Combining soil DNA metabarcoding and ecological parameters to develop a probabilistic mathematical model of fungal species presence

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Background: Wild edible mushroom harvesting is a booming activity in Canada, mostly in Quebec, Ontario, and British Columbia. This increasing demand requires pickers to find more picking areas. However, several challenges are encountered, making fungal inventory difficult to achieve: (*i*) mushrooms are only available during a short period of the year, (*ii*) their abundance varies from year-to-year according to environmental conditions, and (*iii*) only the fructifications are visible. In order to assess the fungal presence of an area, we propose to use environmental metabarcoding combined with next-generation sequencing methods on soil samples. **Results:** The first step of this study was to construct a local DNA barcode reference library for Canadian fungi using a selection of 32 edible species (commercial interest) to be included in a probabilistic presence model. In total, 107 barcodes spanning the entire ITS (Internal Transcribed Spacer) region were obtained from local samples. Soil samples taken from 300 sites, selected among 33 000 sites across the province of Quebec (Canada) that have been characterized by 75 ecological parameters, were sequenced by Illumina MiSeq for the ITS1 and ITS2 region. The metabarcoding data obtained represent the total fungal biodiversity of each site (not only the selected species), and they were combined with ecological parameters to calibrate and generate a mathematical model that can be projected to the 33 000 inventory sites. Presence probability maps have been generated that indicate potential new areas to find the studied species. **Significance:** This is the first effort to use metabarcoding to characterize the fungal biodiversity of soil samples in the province of Quebec and also to conceive a mathematical model to predict the presence of mycelium. It will also provide a key tool to mushroom pickers to facilitate their research across the vast territory of Quebec.

DNA barcodes as a tool for fast biodiversity census and establishment of taxonomic workflows: the case of the mostly unknown moths of Argentina

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Background: Lepidopterans constitute one of the most diverse groups of insects, with a total estimated diversity of nearly half a million species. Most of this diversity is yet to be described and corresponds to species of moths, which represent around 80% of all known species of lepidopterans. In Argentina, there is no official count of moth species, and taxonomic knowledge of this group is scarce. We used DNA barcodes to explore moth diversity in the southernmost region of the Atlantic Forest, a biodiversity hotspot. **Results:** We analyzed 1635 specimens representing 601 taxa (already described Linnaean species or morphospecies determined by us based on external morphology) from 27 families of moths collected in Misiones province. Around half of the specimens were assigned to a morphospecies because we were not able to identify them to species level. All the clustering algorithms implemented (ABGD, TCS, RESL) evidenced the existence of cryptic diversity, with MOTU counts (632-658) always exceeding the number of reference taxa (601). More precisely, we found a great correspondence (around 95%) between already described Linnaean species and MOTU boundaries, but not between these and morphospecies (around 70%). This was because the algorithms merged and split many more morphospecies than Linnaean species. This appears to be a consequence of incorrect morphospecies assignment due to unknown intraspecific polymorphism and sexual dimorphism, and the existence of cryptic species. **Significance:** This study shows that DNA barcodes performed better than the morphospecies approach when dealing with unknown entities. Furthermore, this tool can be used to rapidly delimit putative species that could serve as the foundation for subsequent, more detailed taxonomic studies. At the same time, DNA barcodes can accelerate biodiversity census and the uncovering of cryptic diversity in poorly known groups, like the moths of Argentina.

Barcoding the butterflies of Argentina: species delimitation efficacy, cryptic diversity, and geographic patterns of divergence

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Background: Lepidopterans constitute one of the most diverse groups of insects, with nearly 160 000 species worldwide. In Argentina, over 1200 species of butterflies have been described, with the highest diversity concentrated in the Atlantic Forest in Misiones province. We present here our most comprehensive analysis to date of the DNA barcodes of the Argentinian butterflies. **Results:** We analyzed 2161 specimens representing 429 species from 251 genera collected in eight provinces of northeastern and central Argentina. Mean intraspecific distance was 0.31%, being markedly lower than the mean interspecific distance (7.21%). More importantly, the average divergence to the nearest neighbour (6.91%) was 10 times larger than the mean distance to the farthest conspecific (0.69%). In fact, a barcode gap was observed for all species but four, which were the only ones found to be paraphyletic and (or) involved in cases of barcode sharing. Our barcode library allowed us to correctly identify species of butterflies in 96%–99% of the cases, depending on the identification criterion implemented. As of cryptic diversity, all species delimitation algorithms implemented (ABGD, TCS, RESL) delivered molecular operational taxonomic unit (MOTU) counts that were over the number of reference

species with sequences (417), identifying 424–438 genetic clusters depending on the method. RESL (the algorithm used to delimit BINs on BOLD) delivered the highest percentage of MATCHES (93.5%) between species and MOTU boundaries. Finally, these analyses allowed us to identify several cases of both deep intraspecific splits (some of which are associated with geographic structure) and shallow to non-existent interspecific divergence that will be studied in more depth. **Significance:** This study shows that DNA barcodes are extremely useful both for species identification of Argentinian butterflies and the discovery of cryptic diversity. At the same time, our project contributed to increased knowledge on lepidopterans and museum collections in Argentina and provided new species records for the country.

DNA barcoding of the butterflies of Madagascar

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Background: Butterflies are among the most conspicuous and iconic insect groups of Madagascar. In a recent checklist there were 302 described species, but the fauna can now be updated to 329 recognized species, to which needs to be added a large suite of undescribed taxa. **Results:** We obtained 855 barcodes comprising 279 Barcode Index Numbers (BINs), based on extensive sampling of xeric and mesic environments. **Significance:** This is the most comprehensive DNA barcode database of Malagasy butterflies to date. This enables us to revise traditional taxon limits, cast local species in a global context, recharacterize local species radiations and species endemism levels, and provide a baseline for future field and museum research relying on morphological and molecular identification of species including invasives. It places butterflies in a much stronger perspective for conservation efforts based on multiple taxon inventories and species mapping, as well as redefining species units as a contribution to local species richness and turnover analyses.

The Metabarcoding and Metagenomics Journal: innovative scholarly publishing in a rapidly expanding research field

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High-throughput sequencing (HTS) technologies have opened new avenues for the study of biodiversity. This innovation has resulted in an exponentially increasing number of concepts, methods, and research studies in the fields of metabarcoding and metagenomics (MBMG). Furthermore, studies applying novel MBMG approaches are often accompanied by large data files. While research programs are being transformed by HTS, scientific journals are unable to catch up with the fast pace of developments in this field. The publication of papers in nonmachine-readable format (e.g., paper/PDF) hampers research progress by creating obstacles to the interoperability and re-use of scientific data. In response to the increased interest in molecular biodiversity, ecology, and evolution, as well as in DNA-based monitoring, Pensoft will apply its extensive experience as a technological innovator and open access advocate to launch a conceptually and technologically innovative open science journal, *Metabarcoding and Metagenomics* (MBMG). Featuring novel article formats and data publishing workflows, MBMG will reflect the rapid growth in the use of metabarcoding and metagenomics in the life and environmental sciences. The journal will cover diverse topics including environmental, microbial, and applied MBMG (e.g., biomonitoring, quarantine, environmental assessment, nature conservation, eDNA, species invasions), and associated topics such as molecular ecology, DNA-based species delimitation and identification, and other emerging fields related to MBMG. Submissions of bioinformatic approaches to MBMG (algorithms, software) are also encouraged. MBMG is published on the high-tech ARPHA journal publishing platform, which is the first workflow to support the full life cycle of a manuscript, from writing through submission, peer review, publication, and dissemination within a single online collaborative platform. The XML-based workflow used by the journal ensures that content and data are available for extraction, indexing, and re-use immediately following publication.

DNA-based aquatic bioassessment and monitoring in Europe and beyond: The EU COST Action DNAqua-Net paves the way from research to application

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The protection, preservation, and restoration of aquatic ecosystems and their functions is of global importance. In Europe, it became legally binding mainly through the EU–Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD), in the United States through the Clean Water Act (CWA). In order to assess the ecological status of a given water body, aquatic biodiversity data are obtained and compared to a reference water body of good quality. The quantified mismatch thus obtained determines the extent of potential management actions. The current approach to biodiversity assessment is based on morphotaxonomy. This approach has many drawbacks, such as being time consuming, limited in temporal and spatial resolution, and error-prone due to variation of individual taxonomic expertise. Novel genomic tools, such as metabarcoding and metagenomics, can overcome many of the aforesaid problems and complement or even replace traditional bioassessments. Even biotic components that are currently not used (e.g., many protists) can be included in such novel bioassessments. A central limitation at the moment, however, is the plethora of field and laboratory approaches as well as the lack of conceptual concerns such as the difficulty to obtain abundance estimates from metabarcoding data. In order to maximise efficiency in developing DNA-based tools to the application stage, the programme DNAqua-Net, funded by the European Union as a COST Action, has been implemented. DNAqua-Net nucleates researchers across disciplines with the task to identify best-practice genomic tools and novel eco-genomic indices for routine application in biodiversity assessments and monitoring. Furthermore, DNAqua-Net provides a platform for training of the next generation of researchers, preparing them for the new technologies. Jointly with water managers, politicians, and other stakeholders, the group develops a conceptual framework for the standard application of eco-genomic tools as part of legally binding assessments, in Europe and beyond.

Impact of sequencing platform, target amplicon, and OTU-clustering on DNA metabarcoding of mock communities of marine macrobenthos

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Background: Assessment of species composition in macrozoobenthic communities is a key element of biomonitoring in marine and transition waters. DNA metabarcoding provides the opportunity to improve accuracy and throughput of species richness assessments.

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However, data analysis procedures and assignment tools can influence species detection ability. In a previous study, we tested five COI primer pairs in two mock communities (MC1 and MC2), each containing 21 macrobenthic species in different proportions, using a Roche-454 platform. **Results:** Here, we compare the detection success obtained for the same communities with an Illumina-MiSeq platform using (*i*) one of the five COI primer-pairs (ArF2/ArR5) and (*ii*) a primer pair targeting an alternative marker (V4 region of 18S rDNA). Furthermore, in both platforms we tested the impact of species assignment tools using two different approaches: read-based assignment and OTU-based assignment. BOLD and SILVA databases were used, respectively, for taxonomic assignment of COI reads/OTUs and 18S OTUs with ≥97% similarity. Compared to 454, in MiSeq platform the detection success increased in both communities (MC1: 43% vs. 52%; MC2: 43% vs. 62%). Using the V4 region, species-level resolution was only attained for *Lepidochitona cinerea*. Moreover, some taxa were detected solely by V4, demonstrating a tendency to detect preferentially other taxa than target macrobenthic species. Compared to the individual read-based assignments, OTU-based assignments resulted both in a lower detection success of the target species, together with an excess of putative taxonomic units, i.e., multiple OTUs produced for the same species, resulting in an overestimation of species richness. **Significance:** High-throughput sequencing (HTS) platforms with deeper sequencing capacity can improve species detection success. COI performance in macrobenthos detection remained superior to V4, showing higher recovery rates and species-level resolution. To avoid potential operational artifacts, circumvent OTU-clustering, and to improve the performance of HTS-based macrobenthos monitoring, more efforts should be allocated to the completion of reference libraries.

The mystery of muthi: unveiling the identity of bulbous and perennial plants traded at the Faraday Medicinal Market, Johannesburg, using DNA barcoding

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Background: The South African medicinal market generates ~2.9 billion ZAR per annum, providing a fundamental source of income but comes at the expense of the environment. Most plants traded at these markets are harvested from wild resources, resulting in noticeable levels of species depletion. Adulteration, trade using vernacular names, and morphological similarities of plants, or lack thereof, make identifying samples on a taxonomic level challenging. In this study, DNA barcoding was implemented to rapidly identify bulbous and perennial plants traded at Faraday, South Africa. A list of species traded, including their conservation status, was compared against a known published checklist. **Results:** Sixty samples were collected and sequenced for the core barcoding regions. Three identification methods were used namely, BLAST (Basic Local Alignment Search Tool), Tree-based, and BRONX (Barcode Recognition Obtained with Nucleotide eXposés), permitting 76%, 64%, and 88% of the samples to be identified to species level, respectively. When comparing the final vernacular identities to the proposed scientific names, 20% of the samples matched to species level and 17% matched to genus level. Of the samples, 37% did not link to proposed scientific names, although sharing the same vernacular names, and 26% of the given vernacular names had no proposed scientific names. **Significance:** The high level of disagreement between the vernacular and scientific names indicate instances of misidentification or substitution, emphasising the eminent threat of health risks for end users. Results from this study reveal a noticeable increase in the number of species traded with the majority of sought-after species being of Least Concern. However, 10% of the species are Declining or Near Threatened in the wild, posing serious conservation issues. A prolonged unsustainable trade of these plants could lead to more Critically Endangered species status in future.

Museum harvesting in major natural history collections

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Background: Large natural history collections are a crucial resource of diverse and rare specimens, but their genetic reserves are underutilized. For the last decade, the Centre for Biodiversity Genomics (CBG) has worked to reverse this trend and has set the standard for DNA barcode-based museum processing pipelines. Past efforts were often limited by specimen age, preservation method, and ultimately DNA quality. However, recent advances in high-throughput sequencing (HTS) technologies have made it possible to amplify and sequence DNA from old and rare specimens, even with very limited quantities of DNA. CBG's partnership with the National Museum of Natural History (NMNH) in Washington DC is an exemplar of this system, which incorporates both Sanger- and HTS-based methods to maximize sequencing success rates based on predicted DNA quality. **Results:** To date, over 120 000 specimens from the NMNH have been DNA barcoded and deposited in the Barcode of Life Data System (BOLD). The current focus of this partnership remains on building the barcode reference library of North American Lepidoptera, which is nearing completion. However, efforts have recently expanded to include barcoding the world genera of Lepidoptera, where significant progress can be made at the NMNH due to its vast archives of authoritatively identified material from around the globe. In parallel, the CBG is also working towards completing barcode coverage for every insect family with the assistance of the NMNH. **Significance:** While contributing valuable digitization services to participating institutions, the CBG's museum harvesting pipeline is also producing an invaluable reference barcode library for BOLD, and for the community as a whole. This resource is of critical importance for parameterizing the BOLD Identification Engine, and it will undoubtedly assist its users with the discovery of new, rare, and exciting taxa.

The application of DNA barcoding for the identification of invertebrates, at the Ministry for Primary Industries, New Zealand

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Background: Since DNA barcoding was proposed in 2003, its application for the identification of invertebrates has increased dramatically. It is now widely used as an effective tool that enables rapid and accurate identification of invertebrates for diagnostic purposes. **Results:** Since 2008, DNA barcoding has been applied for routine identification of border interceptions and surveillance samples by the entomology team at Plant Health & Environment Laboratory (PHEL), Ministry for Primary Industries (MPI), New Zealand (NZ). DNA barcoding has provided species-level identification for immature stages and damaged specimens where identification with morphological characters was impossible. DNA barcoding was applied by PHEL for the following: (*i*) Border interceptions: Since 2015, around 500 individual specimens were barcoded each year, which has greatly assisted in the identification of the immature stages of intercepted organisms. Each species-level identification allows the assignment of accurate regulatory status, thus reducing fumigation, which is beneficial to both importers and environment; (*ii*) General surveillance: A DNA barcoding reference library was constructed for samples collected from various locations in NZ. To date, this library includes around 400 sequences, with novel DNA barcode sequences endemic to NZ, building up baseline information for NZ species, and providing a reference for identifying new to NZ species; (*iii*) DNA barcoding

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library construction: DNA barcoding libraries were constructed with morphologically validated specimens from the families Bostrichidae (28 species) and Cerambycidae (16 species). This helps with diagnostics and decision making with respect to intercepted organisms at NZ borders. **Significance:** The application of DNA barcoding has improved the diagnostic capability within MPI. The studies enrich the DNA barcoding reference databases and provide novel information for NZ species. This also highlights the merit of combining morphological and molecular identification, as well as aiding informed decision making at the border, preventing exotic pests crossing into NZ.

DNA barcoding reveals patterns of species diversity among northwestern Pacific molluscs

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Background: The marine molluscs present a significant challenge for morphological approaches to specimen identification because they exhibit differences in life stage, frequently have morphologically cryptic taxa, and substantial phenotypic plasticity, which hampered the conservation and management of the richest diversity of this taxa. In this sense, reliable specimen identification and biodiversity monitoring of organisms in the field is quite necessary. **Results:** In total, 2801 DNA barcodes belonging to 569 species from China, Japan, and Korea were analyzed. An overlap between intra- and interspecific genetic distances was present in 71 species. We tested the efficacy of this library by simulating a sequence-based specimen identification scenario using Best Match (BM), Best Close Match (BCM), and All Species Barcode (ASB) criteria with three threshold values. BM approach returned 89.15% true identifications (95.27% when excluding singletons). The highest success rate of congruent identifications was obtained with BCM at a 0.053 threshold. The analysis of our barcode library together with public data resulted in 582 Barcode Index Numbers (BINs), 72.2% of which were found to be concordant with morphology-based identifications. In the neighbour-joining phenogram, 2320 (83.0%) queries formed 355 (62.4%) species-specific barcode clusters, allowing their successful identification. Thirty-three (33) species showed paraphyly and haplotype sharing. Sixty-two (62) cases are represented by deeply diverged lineages. **Significance:** This study represents the first comprehensive molecular assessment of northwestern Pacific molluscs, and suggest an increased species diversity in this region, highlighting taxonomic revision and conservation strategy for the cryptic complexes.

Combining barcodes and genomics reveals mito-nuclear discordance in the evolutionary history of a widespread passerine (*Troglodytes aedon*)

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Background: COI-based analyses using barcode libraries often discover divergent intraspecific lineages, which are frequently interpreted as having limited gene flow. However, to confirm this one should use nuclear markers, which are better suited for studying gene flow and can currently be obtained at a large scale using genomic techniques. Here, we combine mitochondrial and genomic data (ddRADseq) to study the evolutionary history of the house wren, *Troglodytes aedon*, focusing on the southern cone of South America. **Results:** COI data from around 90 specimens from Argentina, Bolivia, and Uruguay revealed the presence of at least three continental lineages with up to 5% divergence. These lineages, however, were not geographically structured: only one lineage is present in Patagonia, but representatives from two or three different lineages can be found in specific localities in northern and central Argentina (i.e., lineages are largely sympatric in some areas). Our genomic analysis of thousands of markers did not differentiate these lineages and instead suggested high levels of gene flow among mitochondrial clades, with the sole exception of a few birds from northern Bolivia. The subtle nuclear differentiation among mitochondrial lineages could be partially explained by isolation by distance. These results suggest the presence of divergent mitochondrial lineages in a largely panmictic population. This pattern could be due to ancient isolation by barriers that are no longer effective and (or) the consequence of a widely distributed species with a very large effective population size that retains mitochondrial diversity. **Significance:** By showing contrasting mitochondrial and genomic patterns of diversification in a widespread passerine, this study highlights that analyzing nuclear and mitochondrial data together allows for a more complete understanding of evolutionary history. These results also emphasize the need for precaution when mitochondrial patterns are interpreted on their own in evolutionary analyses.

A checklist of the bats of Peninsular Malaysia and progress towards a DNA barcode reference library

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Background: Several published checklists of bat species have covered Peninsular Malaysia as part of a broader region, and (or) in combination with other mammal groups, while other researchers have produced comprehensive checklists for particular localities. To our knowledge, a comprehensive checklist of bats specifically for the entire geopolitical region of Peninsular Malaysia has never been published, yet knowing what species are present in Peninsular Malaysia and their distributions across the region are crucial in developing suitable conservation plans. **Results:** Our literature search revealed that 110 bat species have been recorded for Peninsular Malaysia. For 18 of these species, the latest records from Peninsular Malaysia pre-date the year 2000. Seven species were recorded only once, whereas records for three species lack precise locality information. Our search on the Barcode of Life Data System (BOLD) found 86 (78%) matching taxonomic names with published DNA barcode information. Of these, 48 (44%) species have records originating from Peninsular Malaysia. **Significance:** Neighbour-joining tree analyses of published DNA barcodes and their allocation to Barcode Index Numbers (BINs) on BOLD suggest that several species are composed of two or more distinct haplogroups, which may represent distinct taxa. We discussed these cases in detail and highlight the importance of further surveys and revisions to upgrade the nomenclature used, clarify distributional records, and resolve the taxonomy of particular bat species in Peninsular Malaysia, in order to determine their conservation priorities.

DNA barcoding of alien Ponto-Caspian amphipods from the Belarusian part of the Central European invasion corridor

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Background: Invasive alien species (IAS) are considered an important cause in global biodiversity loss, and their role was recognised by the Convention on Biological Diversity that included IAS among the Aichi Biodiversity Targets to be reached by 2020. For fast and accurate identification of IAS, DNA barcoding arises as an optimal method. Here, we focus on alien Ponto-Caspian amphipods in the Belarusian part of the Central European invasion corridor and their identification through DNA barcoding. Nine species of Ponto-Caspian amphipods are known to have invaded the water bodies of Belarus: *Chelicorophium curvispinum*,

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Chelicorophium robustum, *Dikerogammarus haemobaphes*, *Dikerogammarus villosus*, *Echinogammarus ischnus*, *Echinogammarus trichiatus*, *Obesogammarus crassus*, *Obesogammarus obesus*, and *Pontogammarus robustoides*. Despite a well-developed checklist of alien amphipods for Belarus, the presence of additional IAS (such as *Echinogammarus trichiatus*, *Dikerogammarus bispinosus*, *Chelicorophium mucronatum*, or *Chaetogammarus warpachowskyi*) might go undetected due to misidentification (especially in early developmental stages) or cryptic speciation. **Results:** Barcodes were obtained from 395 specimens, representing nine species (100%) of alien amphipods of Belarus. Mean intraspecific divergences were 0.16%, while average congeneric sequence divergences were 20.35%. Barcode Index Number (BIN) assignments corresponded perfectly with current species boundaries in 89% of these species. Amphipods identified as *E. ischnus* were separated into two BINs. Deep divergences (>3%) were noted for *C. curvispinum*: mean intraspecific distance was 2.40%, while maximum intraspecific distance reached 5.89% (an indication of potential cryptic species). DNA barcoding confirmed the misidentification of *D. bispinosus* and the presence of *E. trichiatus* in Belarus. **Significance:** This is the first effort to provide a molecular inventory of alien Ponto-Caspian amphipod species known from the Belarusian part of the Central European invasion corridor. Moreover, the results of this study will be used to update the national checklist of alien amphipods of Belarus.

A global comprehensive DNA barcode reference library of yews and its forensic applications

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Background: The rapid and accurate identification of species listed in CITES and IUCN is the central issue of bio-surveillance, legal justice, and conservation. However, the need is dependent on a comprehensive DNA barcode reference library with extensive sampling at taxonomic and geographical scales. Here, we select *Taxus* as a model to construct a comprehensive DNA barcode library of all yew species worldwide to show its application in three forensic cases. **Results:** For the library construction, first, we used multiple species delimitation methods to assess the five proposed DNA barcodes and their combinations with Dataset I including 72 individuals representing all the 15 species of *Taxus*; second, we re-evaluated the confidence of the five barcodes using an extended Dataset II with varied sample size from 110 to 195 individuals for each barcode; finally, we merged the phylogeographical data to construct a haplotype-based reference library of barcode marker *trnL-F* in Dataset III, comprising a total of 3763 trees of yew. Then we applied the DNA barcode reference library to identify *Taxus* samples associated with three forensic cases, which provided robust evidence for accurate species identification and natal inference. **Significance:** The DNA barcode reference library of global yew species developed here will be important for species identification and illegal trade monitoring of endangered species of *Taxus* listed in CITES and IUCN, as well as the application of bio-surveillance and conservation management of endangered yews.

DNA barcoding evaluation and implications for phylogenetic relationships in Lauraceae from China

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Background: Lauraceae are an important component of tropical and subtropical forests and have major ecological and economic significance. Owing to a lack of clear-cut morphological differences between genera and species, this family is an ideal case for testing the efficacy of DNA barcoding in the identification and discrimination of species and genera. In this study, we evaluated five widely recommended plant DNA barcode loci *matK*, *rbcL*, *psbA-trnH*, ITS2, and the entire ITS region for 409 individuals representing 133 species, 12 genera from China. We tested the ability of DNA barcoding to distinguish species and as an alternative tool for correcting species misidentification. We also used the *rbcL+matK+psbA-trnH*+ITS loci to investigate the phylogenetic relationships of the species examined. **Results:** Among the gene regions and their combinations, ITS was the most efficient for identifying species (57.5%) and genera (70%). DNA barcoding also had a positive role for correcting species misidentification (10.8%). Furthermore, based on the results of the phylogenetic analyses, Chinese Lauraceae species formed three supported monophyletic clades, with the *Cryptocarya* group strongly supported (PP=1.00, BS=100%) and the clade including the *Persea* group, Laureae, and *Cinnamomum* also receiving strong support (PP=1.00, BS=98%), whereas the *Caryodaphnopsis–Neocinnamomum* received only moderate support (PP=1.00 and BS=85%). **Significance:** This study indicates that molecular barcoding can assist in screening difficult to identify families like Lauraceae, detecting errors of species identification, as well as helping to reconstruct phylogenetic relationships. DNA barcoding can thus help with large-scale biodiversity inventories and rare species conservation by improving accuracy, as well as reducing time and costs associated with species identification.

A molecular clock for Arctic marine invertebrates

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Background: Divergence times for Arctic marine lineages have commonly been estimated using calibrations from geographically distant taxa. However, due to evidence of rate heterogeneity among taxa and environments, it is essential to pursue clock calibrations for Northern lineages. The opening and re-closure events of the Bering Strait provide an exceptional resource for calibrating the molecular clock in Northern marine taxa. Here, we used the novel "iterative calibration" approach to incorporate the complete glacial history of the Bering Strait for clock calibration. Using publicly available sequences of the cytochrome *c* oxidase subunit I (COI) gene, we explored patterns of molecular divergence across 91 trans-Bering sister clades of marine invertebrates. **Results:** Kimura 2-parameter (K2P) divergences between trans-Bering sisters ranged from 0.12% to 26.37%. Assuming simultaneous isolation of all trans-Bering pairs during the major trans-Arctic interchange (3.5 Ma), as commonly assumed in the literature, would imply high variability in evolutionary rates. However, rate heterogeneity was not the major explanation since the molecular clock hypothesis was rejected for only five pairs, and whole-tree analyses for select taxa indicated only modest clock deviations. Thus, the results strongly support previous research suggesting multiple pulses of trans-Bering migrations. Our results also suggest a rate of K2P divergence of 2.8%/MY in echinoderms, 3.2%/MY in molluscs, 3.5%–4.7%/MY in polychaetes, and 5%–5.2%/MY in arthropods. **Significance:** Interestingly, our results contrast with a highly cited low divergence rate reported for tropical lineages (1.4%/MY), but they agree with several other published calibrations (3%–5%/MY). However, by integrating genetic, biogeographic, and fossil evidence, and using a substantial number of sister clades, we anticipate more accurate calibrations than when using

simplistic assumptions. The new rates of molecular evolution presented here will advance our ability to date recent evolutionary events in the marine realm and will expand our understanding of the impacts of prior climatic changes upon the history of life.

Assessing a DNA mini-barcode strategy for species identification in neotropical necrophagous blow flies (Diptera: Calliphoridae) of forensic importance

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Background: Higher Diptera exhibit overwhelming variety in terms of morphology, habits, and species diversity. Among dipterans, the necrophagous Calliphoridae (blow flies) are the most important insects commonly used in the forensic entomology framework. Because of underestimated assessed diversity in the Neotropical region, species identification, and delimitation of biological entities become a difficult task based on morphological characters alone. Studies using the standard COI barcode region have given rise to controversial results and challenges in obtaining amplicons from blow flies and old specimens. **Results:** A 331-bp fragment was analyzed and used as an alternative mini-barcode in 150 specimens, including species from 12 genera of forensic importance: *Calliphora*, *Blepharicnema*, *Chlorobrachycoma*, *Chloroprocta*, *Chrysomya*, *Compsomyiops*, *Lucilia*, *Paralucilia*, *Hemilucilia*, *Cochliomyia*, *Sarconesiopsis*, and *Roraimomusca*. Distance matrix and dendrograms revealed useful polymorphisms to cluster specimens at the generic, and in some cases at the species, level. Clusters confirmed the currently proposed classification at the generic level based on morphological characters. Some species of *Lucilia*, *Paralucilia*, and *Hemilucilia* were not monophyletic. For the first time, molecular data for *Chlorobrachycoma* were obtained. **Significance:** The use of DNA mini-barcode is an alternative to achieve COI sequences from blow flies, including those from specimens with more than 50 years of storage. The short COI sequences allowed a reliable assignment mostly at the generic level, and partially at species level. Our approach provides a useful backbone to DNA mini-barcode dataset for Neotropical flies.

Advancing DNA barcoding applications: monitoring environmental, agricultural, and public health outcomes

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DNA barcoding has come a long way in a relatively short period of time. Some of the most exciting applications of DNA barcoding are the potential to identify species and biological products across a broad range of previously intractable situations. However, we still have some way to go to further develop the approach to harness the latest sequencing capabilities and ensure that DNA barcoding remains at the cutting edge. This talk will outline a range of DNA barcoding applications through case study examples and the steps that are now being taken to embed DNA barcoding in standardised screening frameworks and policy, including (*i*) control of illegally logged and non-conforming timber products in global supply chains, (*ii*) monitoring the return of ecological function to restored ecosystems, (*iii*) revealing ecological relationships between taxa to augment agricultural decision making, (*iv*) understanding the benefits of green space exposure to the human microbiome and public health, and (*v*) development of a human microbiome fingerprint. The talk will finish by outlining recent advances and options for cheap, quick multi-locus DNA barcoding approaches along with the challenges this will bring to the barcoding community in terms of standardisation and data management.

Assessing the efficacy of DNA barcoding in Protura (Arthropoda: Hexapoda)

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Background: Protura, a poorly known group of tiny soil animals, is one of the most primitive hexapods. Based on morphological taxonomy, there are more than 800 known species in Protura that have been described in 10 families of 3 orders. However, most diagnostic characters are difficult to recognize due to their small body size (~1 mm), and only a select number of scientists can identify the proturan species in the world, which has seriously impeded the studies on the phylogeny and ecology of Protura. This study aimed to examine if the DNA barcoding (COI gene) is a useful approach for determining proturan species. **Results:** The study sequenced and analyzed DNA barcodes of 265 proturan specimens from 61 species belonging to 26 genera, 8 families, and 3 orders. For most species, the molecular clusters are well consistent with the morphological determination. However, some unusually large intraspecific genetic distances (up to 25%) may indicate the presence of cryptic species. In addition, our data show low genetic variation within populations, but reveal high genetic differentiation among different geographic populations, with a notable correlation between geographic and genetic distances. **Significance:** The study clearly demonstrates that the standard DNA barcoding is effective but not enough for species discrimination of Protura. The taxonomy and biogeography of Protura are worth further studies by using more molecular markers.

Using DNA metabarcoding to reveal the role of hoverflies (Syrphidae) in pollen transport

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Background: Pollination by insects is a key ecosystem service, but there is concern about the decline in pollinators, caused by habitat degradation, diseases and parasites, and climate change. There are key gaps in the pollination science evidence-base, particularly relating to which insects pollinate which plants, and how pollination networks are structured. Most research has focused on bee populations. However, hoverflies (Syrphidae) also feed exclusively on nectar and pollen, making them potential pollinators of a wide range of wild plants and crops. **Results:** Using DNA metabarcoding to identify pollen, we investigated pollen transport by a range of hoverfly species in conservation grasslands in Wales, UK. It was possible to assign over 98% of sequences to species, genus, or family level. Hoverflies transport pollen from more plant species than previously appreciated. Networks are generalised at the site and species level, but at the individual level varied from specialised to relatively generalised. This suggests that generalised networks may result from a series of short-term specialised feeding bouts by individual insects. Most pollen recorded came from common plant taxa. However, differences in the proportions of various plant taxa in pollen loads between hoverfly species demonstrate some functional complementarity. **Significance:** We show the value of DNA metabarcoding in investigating plant–pollinator interactions. It allows the systematic investigation of pollination networks, from individual insects to whole communities. Our results show how generalised networks can emerge from the short-term specialisation of individuals, thus reconciling generalised network structures with effective plant pollination. Treating hoverflies as a single functional group underestimates the range of pollination function within this ecologically diverse guild. This study is one

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of the first to use DNA metabarcoding to investigate a pollinator community and adds to our understanding of the role of hoverflies in pollen transport.

Barcoding of bats (Order: Chiroptera) in the Philippines using the mitochondrial cytochrome *c* oxidase subunit I gene

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Background: The Philippines serve as a habitat for at least 206 native species of terrestrial mammals, more than half of which are endemic to the country. From these, 78 bat species (order Chiroptera) have been described, with 26 identified as endemic. Scientific data on chiropterans is far from complete, and threats endanger many chiropteran populations. These problems necessitate the development of proper conservation and management measures, which can only be formulated to fit specific needs of certain species with enough knowledge about the taxon of concern. DNA barcodes facilitate the process of identification by providing an accurate, rapid, and more effective method of species recognition. **Results:** A 598-bp portion of the cytochrome *c* oxidase subunit 1 gene was sequenced from 130 individuals belonging to 33 species from chiropteran familes Pteropodidae, Rhinolophidae, Molossidae, Megadermatidae, Hipposideridae, Emballonuridae, and Vespertilionidae to create barcode records for these taxa. Neighbour-joining (NJ) trees of the COI sequences from this study and from GenBank and the Barcode of Life Data System (BOLD) was able to discriminate most species within each family. Several rhinolophid species, *Rhinolophus arcuatus*, *R. inops*, and *R. subrufus*, had low interspecific distances (<2%), and COI was not able to differentiate between them. Inspection of the NJ tree revealed distinct Philippine lineages of species recorded also from other countries as well as possible cryptic species within the different islands. **Significance:** This study generated new DNA barcodes for a more robust barcode data base that will aid environment officers in bio-monitoring and wildlife forensics. Results from the tree provides information about unique species lineages that can be further investigated by further studies using more comprehensive techniques, such as next-generation sequencing.

DNA metabarcoding of benthic chironomid larvae of the Baltic Sea for monitoring of environmental status, biodiversity, and ecological studies

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The EU Water Framework Directive and the Marine Strategy Directive require that European states monitor the ecological status of the aquatic environment and take management actions to ensure that good ecological and environmental status is achieved. The status is assessed by regular monitoring of certain indicators, such as benthic invertebrate macrofauna of various environmental sensitivity. The Baltic Sea, surrounded by range states populated by roughly 90 million people, is heavily affected by, e.g., eutrophication, leading to areas of hypoxic and anoxic sediments. Chironomid larvae are important indicator species in these waters, and can make up about 30% of the macrozoobenthos biomass, which makes them ecologically important as, e.g., fish food. However, many species (<10%) cannot be identified as larvae by traditional means. Thus, for the management authorities we developed (*i*) a local COI barcode reference database of about 1000 specimens of 150 species, which include the vast majority of those with larval stages in the coastal benthos, and virtually all species that were sampled can be identified by these barcodes; and (*ii*) an NGS metabarcoding system based on a 313 bp of the COI to be matched against the reference database. Results will be presented from the metabarcoding of pooled samples from a subset of 20 benthic samples collected in the routine aquatic environmental monitoring program in the Gulf of Bothnia, and how species composition varies among stations with varying environmental conditions. Also, examples of other ecological applications will be shown and discussed. The system enables substantial improvement for taxonomic resolution, quality of environmental indicator information, and increased knowledge of the diversity of these taxa and their ecological role.

DNA barcoding of non-native succulent plants in the horticultural trade industry in South Africa

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Background: Horticultural trade has been identified as a key pathway through which non-native species are introduced into new environments globally. Among the non-native-species, succulent plants have been increasingly prioritised, despite the negative ecological and economic impacts they pose to local native diversity. Although strict laws exist in South Africa governing the trade in biological materials, current measures to regulate this trade are insufficient. This is due, in part, to challenges intrinsic to species identification, and the lack of reliable standards with which traded species can be compared. Here, we seek to use the core DNA barcode regions (*rbcLa* and *matK*) to create a reference library of all traded succulent species in South Africa to facilitate rapid and accurate identification. The potential risk of the horticultural trade in the spread of alien species in South Africa is also evaluated. **Results:** Of the 73 succulent plant species commonly listed in trade in South Africa, 38% had DNA barcode data available on the Barcode of Life Data System (BOLD). To complement this list, DNA barcode data were additionally generated for 45 species representing 62% of the total succulents on our list. Furthermore, we found that 63% and 1% of the succulent species have been formally categorized as either invasive or prohibited, respectively. An additional 36% of the species are in need of formal categorization. **Significance:** The results show the efficacy of DNA barcoding as a tool to correctly assign traded unknown succulents to species. We recommend the use of this technique to ensure traders comply with legislature, which is largely being violated in the horticultural trade industry. If implemented, this will help to prevent further environmental and economic challenges posed by non-native species.

Is everything everywhere, all the time? Sampling site and time influence community composition inferred through eDNA metabarcoding of streams

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Background: Environmental DNA (eDNA) has become a popular tool for the assessment of both single species and communities. However, it remains largely unclear how sampling time and sampled microhabitat influence the assessment of communities in rivers via eDNA metabarcoding. Current, flow rate, and microhabitat in streams can differ on small spatial and temporal scales that may greatly influence metabarcoding results. If so, sampling strategies need to be adjusted accordingly. **Results:** In this study, we took water samples from three rivers and four sites each: (1) close to the surface of the left riverbank, (2) close to the surface of the right riverbank, (3) directly beneath the first sampling site, close to the riverbed, and (4) directly beneath the second site, close to the riverbed. For the rivers Ruhr and Möhne, sampling was conducted three times in spring, each sampling one week apart. The rivers Ruhr and Gillbach were again sampled in autumn. Sequencing on Illumina MiSeq with COI primers Bf2/BR2 re-

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vealed diverse communities and allowed "fingerprinting" of rivers, i.e., communities could be undoubtedly assigned to rivers. In the Ruhr, community composition changed significantly over time, while it did not in the Möhne. Sampling site had a significant influence on community composition in Möhne, Ruhr (autumn) and Gillbach based on all operational taxonomic units (OTUs), particularly metazoan OTUs. **Significance:** This is, to the best of our knowledge, the first study on temporal and spatial patterns of eDNA distribution in rivers. As the technique of eDNA metabarcoding is used in a multitude of studies addressing ecological questions as well as for biodiversity assessments, future studies should take into account that not all eDNA is everywhere, and not all the time. Further investigations should address the found spatial and temporal patterns on a broader geographic scale in order to infer general patterns.

eDNA to detect invasive species: uses, limitations, and alternatives

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Detecting rare, native, or introduced (invasive) species is challenging, particularly in aquatic ecosystems. The advent of environmental DNA (eDNA) provides exciting opportunities to detect these rare species. Early reports suggested that eDNA may be more sensitive to the presence of rare species than traditional methods. A key concern, however, is reducing false negatives while constraining false positives. In this presentation, I will examine the use of eDNA versus other advanced (image analysis, GoPro videography) and traditional (netting) techniques, using examples of invasive fish (tench) in South Africa. Detection sensitivity (CPUE) was higher for eDNA than for GoPro or conventional seine netting. However, seine netting yielded higher detection probabilities and occupancy estimates when using iterative presence/absence data in habitat occupancy models. Problems with sensitivity apply to many single-species detection studies. A review of 79 papers revealed that 93% and 94% of conventional PCR and qPCR studies, respectively, did not report whether primers used had been screened (optimized) for detection sensitivity. In addition, in 48% and 83%, respectively, of cases in the same studies, the detection limit was not reported. Both issues increase the possibility of false negatives. Only 12 of 20 aquatic species on the "world's worst invasive species" list have barcodes in Web of Science, and of these only three have been screened for sensitivity. Metabarcoding approaches are increasingly used by researchers to assay total species richness in habitats like lakes and estuaries, yet this approach can suffer from false negatives or false positives depending on the procedures used. Our experiences with 18S suggest that sequences should be as long as possible, sequence quality filtering should be relaxed, clustering should not be performed, and that singletons should be discarded. We expect continued use and refinement of eDNA approaches for both single- and multi-species detection, reducing problems of false negatives and false positives.

Re-opening the case for wild Frankenflora: testing species of *Protea* for hybidization using DNA and HRM analysis

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Background: In 2006, Dr. Tony Rebelo wrote an article entitled "A Hybridization between planted Proteas and their cultivars with wild Proteas: The Frankenflora" as part of the Protea Atlas Project. Since then, the sum total of all papers published directly dealing with the topic of hybridization in wild Proteas is 0 (Note: that there is an extensive list of publications on commercial Protea hybrids!). Inter- and intraspecific hybridization is a highly potential, and entirely understudied, threat to Cape biodiversity. Anthropogenic dispersal of Cape lineages has dire consequences for genetic integrity of many Cape species as was highlighted by Dr. Rebelo more than a decade ago. **Results:** Genetic, morphological, and chemical methods provide a robust case study of hybridization of *Protea eximia* and *P. susannae* in the Van Staadens Flower Reserve, outside Port Elizabeth and together with demographic modelling, can be used to calculate the extent and rate of hybridization across these species in this area. High-Resolution Melt (HRM) screening is used to investigate levels of hybridization. This project uses genetic plant barcoding to generate information on the degree of relatedness of hybrids to the original "pure" parent species. **Significance:** This project aims to get the Frankenflora issue back onto the conservation table (at least in terms of providing robust published scientific evidence). This is a relatively cheap method and so will provide the ability to potentially genetically screen entire populations. The Cape is facing a long list of threats. This project aims to help ensure that our own Eastern Cape species are not included on that list, highlighting the peril by starting off with a straightforward and unexplored study system. In future, a study such as this will be of great importance to the internationally lucrative local cut flower industry in South Africa.

The potential of DNA barcoding as a basis for taxonomic revisions: integrative taxonomy and systematics of *Allodia* Winnertz (Diptera, Mycetophilidae)

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Fungus gnats (Mycetophilidae) is a species-rich family of flies, which has an exceptionally high diversity in northern boreal forests, and there is a clear pattern of trans-Palaearctic and circumpolar species distributions. The taxonomic expertise of the group has long traditions in northern Europe, and the fungus gnat fauna is well known. This accumulated knowledge has been used to build up the database of identified species through The Norwegian Barcode of Life (NorBOL) initiative, which serves as an excellent basis to study large or problematic groups of fungus gnats. It enables the comparison of DNA barcodes across the Holarctic region, and to compare sequences from identified specimens with those of unknown publicly available specimens. *Allodia* is a mycetophilid genus with about 40 species considered present in the Holarctic. Most of the species are described from northern Europe, but expected to be present or have close relatives in North America, although very little is known about the Nearctic fauna. As many as 56 species are recognized based on the Barcode Index Number (BIN) (taxonomic unit) system in the Barcode of Life Data System (BOLD), most from the Holarctic. Hitherto only 21 of these BINs have assigned names. Several of the unidentified BINs stem from Canadian material (deposited in University of Guelph, Centre for Biodiversity Genomics) and are most likely new to science. In this study, the validity of the possible species is investigated based on morphology and additional nuclear DNA sequences. An interesting finding is that several of the unidentified species appears to have sister species limited to the Palaearctic. This will all be a part of a comprehensive review of the *Allodia* s. str., in which the extensive library of DNA barcodes makes the foundation for exploring the species diversity and distribution.

DNA barcoding of *Ficus virens* Aiton (Moraceae) complex present in biodiversity hotspot of South India and its taxonomical implications

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Background: *Ficus*, a complex genus currently circumscribed with ~735 species under 6 subgenera (*Urostigma*, *Pharmacosycea*, *Sycomorus*, *Sycidium*, *Synoecia*, and *Ficus*) in various sections and subsections, is

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distributed in Asia, Australia, Africa, and America. *Urostigma* is the largest subgenus with ~280 species, of which ~44 are in India and ~31 in South India. *Ficus virens* is a complex species belonging to subgenus *Urostigma*, section *Urostigma*, subsection *Urostigma*. It is a polymorphic species and shows variation in shape, size, and nature of leaves, figs, and formation of terminal resting buds. Different forms of species have been explained in nearly 30 different names in earlier floras and created a complex, which is difficult to delimit morphologically. These species also show morphological similarities with its allied species, i.e., *F. lacor*, *F. middletonni*, *F. caulocarpa*, *F. superba*, and *F. concinna*. To resolve the complexity and delimit these taxa, it is inevitable to study the phylogenetic relationship based on DNA markers (eg. ITS2 and *psbA-trnH*) that can be combined with morphological analysis for a final conclusion. **Results:** This study clearly proves that DNA markers have the ability to discriminate species compared with morphological analysis. ITS2 from nuclear and *psbA-trnH* from chloroplast regions showed 100% amplification and sequencing success. This study also examined the intra- and interspecific divergences between the species. The intraspecific divergences were 1% and interspecific divergences were 5%. A phylogenetic tree was constructed using ITS2 and *psbA-trnH* region, which showed 100% species resolution. **Significance:** This is the first effort to compile a reference library of DNA barcodes for the *F. virens* complex of South India that provides species-level identification by taking into account taxonomical inputs. This study has clearly proved that DNA barcoding is a potential tool to distinguish taxonomically complex groups, which will have huge taxonomical implications.

Effect of eDNA filtration strategies on metabarcoding success of freshwater metazoan communities

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Background: Identification of freshwater metazoan communities based on metabarcoding of environmental DNA (eDNA) offers new opportunities for biodiversity assessments. Filtering of water to capture eDNA is superior to precipitation by centrifugation. Because of logistical challenges in transporting large volumes of water, filtering water in the field is advantageous. Appropriate filter selection/preservation is therefore crucial for maximum DNA recovery and sample replicability. **Results:** We studied the effect of two filter types, four different filter preservation techniques, as well as pre-filtration on DNA yield, number of recovered operational taxonomic units (OTUs) from amplicon sequencing, and inferred metazoan community composition, using eDNA collected from a river and a lake ecosystem. Our results showed that 0.45 μm mixed cellulose ester filters yield higher concentration of DNA, higher number of OTUs, and more similar community composition as compared to 0.20 μm polyethersulfone filters. Additionally, pre-filtration negatively affected the DNA yield and OTU numbers but resulted in more similar community composition as compared to direct filtration. Filters preserved either dry or in lysis buffer provided most similar community composition, while ethanol proved a poor preservative for filters. Ethanol-preserved filters recovered fewer OTUs and a more variable community composition than other approaches. **Significance:** Despite vivid research and reviews focused on different issues relating to eDNA sampling and analysis techniques, best practice protocols are still under development. Based on our results, supportive sampling guidelines for community-level eDNA studies may be formulated.

Effect of DNA extraction methods on metabarcoding success of homogenized freshwater macroinvertebrate community samples

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Background: Characterization of freshwater biodiversity based on metabarcoding of homogenized bulk samples offers new opportunities for environmental assessments. Appropriate DNA extraction methods are crucial for maximum DNA recovery and sample replicability. **Results:** We sampled freshwater invertebrates from six river and lake sites. DNA was extracted from homogenized bulk samples using the HotSHOT approach, the Qiagen DNeasy Blood & Tissue Kit, and the MO BIO PowerPlant Pro DNA Isolation Kit (now called Qiagen DNeasy PowerPlant Pro Kit). We measured DNA yield, the number of recovered operational taxonomic units (OTUs), and compared community composition. PCR inhibitors were present in all HotSHOT samples, in 75% of the Blood & Tissue Kit samples, and in 25% of the PowerPlant Kit samples. The PowerPlant Kit resulted in the highest DNA yield, and the Blood & Tissue Kit resulted in the lowest. The number of OTUs was higher in the samples extracted with the PowerPlant Kit than the samples extracted with the HotSHOT method or the Blood & Tissue Kit. However, the recorded community composition was not significantly affected by the different extraction methods. **Significance:** PCR inhibition is a factor that may influence metabarcoding results of homogenized bulk samples. Based on our study, the PowerPlant Kit removes the inhibitors most effectively, and no extra DNA purification prior to amplification is needed, making the workflow from sampling to results simpler.

Assessing DNA barcodes as a diagnostic tool in identification of giant African land snail species

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Background: High rates of loss and species discovery have led to the urgent need for more rapid assessments of species diversity and distribution in the molluscs, an approach now offered through DNA barcoding. Prior DNA barcoding work on snails has revealed higher biodiversity counts than previously estimated due to cases of cryptic and undiscovered species in both classes. Despite past research, these taxa are very much in need of comprehensive species-level coverage. **Results:** This study constructs a reference library of DNA barcodes for giant African snails and assesses their applicability as a technique for species delimitation. This study also examines the correspondence of current species boundaries with the Barcode Index Number (BIN) system. Barcodes were obtained from 500 specimens, representing 100 species of the South Karnataka molluscan fauna. Mean intraspecific divergences were 1% and 3%, while average congeneric sequence divergences were 16% and 14% in amphibians and reptiles, respectively. BIN assignments corresponded perfectly with current species boundaries in 58% of these species. Barcode sharing was observed in four genera of snails, while deep divergences (>2%) were noted in 21% of the species. Using multiple primers and a refined PCR regime, barcode fragments were recovered from a few formalin-fixed specimens, demonstrating that formalin collections can expand genetic databases. **Significance:** This is the first effort to compile a reference library of DNA barcodes that provides species-level identifications for molluscs across a broad geographic area. DNA barcodes from South Karnataka molluscan fauna were used to construct a phylogenetic tree. This study also highlights the merit of further investigation into obtaining genetic material from formalin-fixed tissue and the use of DNA barcodes for biodiversity forensics.

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DNA barcoding technology in Belarus: perspectives and needs

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Background: The Republic of Belarus acceded to 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. To implement its Article 17 "Monitoring the Utilization of Genetic Resources", taxonomic verification of genetic resources should be conducted. DNA barcoding is the best approach and tool to study flora and fauna and to organize the obtained data in a special database. The DNA identification of rare and endangered plant species started in 2017 at the Republican DNA Bank of a Human, Plants, Animals, and Microorganisms to be active and closer to the use of DNA barcoding techniques to assess species diversity and their distribution in the total gene pool of plants. **Results:** Eight rare and endangered plant species are used for research: dwarf Arctic birch (*Betula nana*), cross gentian (*Gentiana cruciata*), wood anemone (*Anemona sylvestris*), large-flowered foxglove (*Digitalis grandiflora*), Siberian iris (*Iris sibirica*), sage meadow (*Salvia pratensis*), blackthorn (*Prunus spinosa*), and European pine (*Abies alba*). Specimens were collected in the National Park "Narochansky" in 2016. The possibility of using DNA barcoding primers for the amplification of chloroplast (*rbcL*) and nuclear (ITS2) DNA regions is being studied. The second study objective is to construct a reference library of DNA barcodes for rare and endangered plant species. **Significance:** The direct analysis of DNA nucleotide sequences and design of phylogenetic trees will allow the enhancement of traditionally used systems of classification. This is the first effort in Belarus to compile a reference library of DNA barcodes to provide the identification of rare and endangered species of plants across National Parks and to detect errors or any divergences in taxa identification. Scientific collaboration and training on the uses of DNA barcoding techniques are in dire need for specialists in Belarus.

DNA barcoding and molecular systematics of *Searsia*

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Background: The family Anacardiaceae includes ~800 species in 82 genera. *Rhus* L. is the largest and most widely distributed genus in Anacardiaceae. However, controversies have surrounded species delimitation in this genus. *Searsia* F.A. Barkley was originally included into the *Rhus* complex, but recent molecular studies have separated it along with six other genera from the *Rhus* complex and recognised them as separate genera. The genus *Searsia*, which is the focus of the current study, is represented by ~120 species and is widely distributed in Africa with only three species currently known from Asia. Here, we included an extensive sampling and molecular analyses of species of *Searsia* across its distribution range in Africa to access the relationships within the genus. **Results:** In total, 205 taxa, representing 35 species of *Searsia*, were sequenced for the core barcoding regions (*matK* and *rbcLa*) along with additional markers (i.e., ITS, ETS, *trnL-F* and *ndhF*). **Significance:** This study contributes a first large sampling of *Searsia*. An infrageneric classification for the genus will be presented.

Unfolding global biodiversity patterns of marine planktonic diatom communities across the world's oceans

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Background: Analysis of microbial eukaryotic diversity is fundamental to understanding an ecosystem's structure, biology, and ecology. Diatoms (Stramenopiles, Bacillariophyceae) are one of the most diverse and ecologically prominent groups of phytoplankton. This study was performed to enhance the understanding of global biodiversity patterns and structure of planktonic diatom communities across the world's oceans. We used the metabarcoding data set generated from the biological samples and associated environmental data collected during the Tara Oceans (2009–2013) global circumnavigation covering all major oceanic provinces. **Results:** A total of ~18 million diatom V9-18S rDNA tags from 126 sampling stations, constituting 631 size-fractionated plankton communities, were generated. Using ~250 000 unique diatom metabarcodes, the global diatom distribution and diversity across size classes, genus, and ecological niches was assessed. Notably, our analysis revealed (*i*) a new estimate of the total number of planktonic diatom species, (*ii*) a considerable unknown diversity and exceptionally high diversity in the Open Ocean, and (*iii*) complex diversity patterns across oceanic provinces. Also, co-occurrence of several ribotypes in locations separated by great geographic distances (equatorial stations) demonstrated a widespread but not ubiquitous distribution. **Significance:** This work provides a comprehensive perspective on diatom distribution and diversity in the world's oceans and elaborates interconnections between associated theories and underlying drivers. It shows how metabarcoding approaches can provide a framework to investigate environmental diversity at a global scale, which is deemed as an essential step in answering various ecological research questions. Consequently, this work also provides a reference point to explore how microbial communities will respond to environmental conditions.

Barcoding of Kuril-Kamchatka (NW Pacific) deep-sea amphipods

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Background: The amphipod fauna of the deep-sea is poorly known with only 400 benthic species found below 2000 m worldwide, of which 50 were recorded from the abyssal of NW Pacific. Deep-sea invertebrates, especially crustaceans, are very fragile and can be easily destroyed during sampling. Thus, obtaining proper morphological material is difficult. This makes DNA barcoding a precious tool for biodiversity assessment of deep-sea organisms. We studied diversity of deep-sea amphipods from the Kuril-Kamchatka Trench and the adjacent abyssal plain. The material was collected using epibenthic sledge at 21 stations, along ~1000 km transect, at the depth range of 4830–8745 m. **Results:** The identified amphipods belong to 22 families and were preliminarily assigned to 65 morphospecies. Most importantly, almost half of the species are potentially new to science. So far, we obtained 472 DNA barcodes divided into 130 Barcode Index Numbers (BINs). Up to 7 BINs were recognized within a morphospecies, indicating existence of potential cryptic species. **Significance:** This is the first such wide study dealing with biodiversity of deep-sea Amphipoda at the molecular level. Taking into account that, after Polychaeta and Isopoda, amphipods are among the most abundant abyssal invertebrate groups, the knowledge upon their real diversity is a first step towards understanding the functioning of the deep-sea ecosystem.

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Towards the DNA barcode reference library for European freshwater crustaceans. A summary of recent efforts

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Background: The factual diversity of European freshwater crustaceans is scarcely known. This is particularly surprising taking into account their wide applicability in biomonitoring as well as in ecotoxicological and phylogeographical studies. Crustacea, particularly Malacostraca, play a fundamental role in functioning of freshwater ecosystems, being key taxa in biodiversity conservation. On the other hand, several species are known to be successful invaders posing a threat on local communities. Recent molecular studies on decapods, amphipods, isopods, and mysids show that their real diversity greatly exceeds the number of already described morphospecies. Yet, its large-scale spatial pattern and onset are still weakly understood. **Results:** So far we have obtained more than 8000 COI DNA barcodes for malacostracan crustaceans from all over Europe and combined them with barcodes already existing in the Barcode of Life Data System (BOLD) and GenBank. We covered the traditionally recognized Mediterranean biodiversity hotspots, the northern post-glacial regions, lowland and alpine areas, lakes, rivers, and spring systems. We discovered presence of very high cryptic diversity and numerous molecular operational taxonomic units (MOTUs) endemic for mountain ranges, even in relatively high latitudes, as well as for more southern lowland areas. On the other hand, we observed the presence of several widespread MOTUs with dynamic demography in post-glacial regions. With the aid of other molecular markers we could attribute the observed diversity patterns, even at species level, to a series of geological and climatic processes ranging from Neogene to the retreat of Pleistocene Ice Sheet as well as to very recent anthropogenic factors. **Significance:** Our study is a first attempt to construct a comprehensive and publicly available COI DNA barcode library for freshwater malacostracan crustaceans in Europe. Proper identification of MOTUs, as well as mapping and understanding their distribution, is of utmost importance when using crustacean models in evolutionary/ecological studies, biomonitoring, and risk assessments.

Unmasking the succulent plant trade at the Faraday traditional medicinal market in Johannesburg, South Africa

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Background: The Faraday traditional medicinal market is dedicated to the trade of plant and animal material used in traditional African medicine ("muthi"). The materials traded are accessible and affordable to most African communities and are often preferred over western medicines, due to poverty. Quantifying the illegal trade of succulent plant species at the Faraday medicinal market has not yet been undertaken exhaustively. Most succulents are commonly used to treat ailments such as arthritis, eczema, and constipation. However, it remains challenging to distinguish between the various succulent species at the market due in part to the fact that a majority of the species are being sold as either dried, crushed, or withered. Here, DNA barcoding was used to identify succulent plants currently traded at the Faraday market. Plant samples were collected and sequenced using the standard DNA barcoding regions (*matK* and *rbcLa*). **Results:** Two identification methods, BLAST and the tree-based, show that the majority of traded succulents are from the families Aspholedaceae, Crassulaceae, Mesembryantheaceae, Portulaceae, and Euphoriaceae. **Significance:** This study provides an important list of succulent plant species currently traded at the Faraday market that are endangered or likely to become endangered due to over-exploitation and highlights the importance of sustainable management of wild medicinal plants sold at the market.

Genomic barcoding of plants: African trade in *Anacyclus* (Asteraceae)

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Background: Increased grazing related to population growth and unsustainable harvesting due to higher international demand threatens the survival of wild populations of medicinal plants as well as the local livelihoods dependent on this trade. Pellitory is a popular remedy from the Atlas Mountains of Morocco used in Arab traditional medicine and Indian Ayurvedic medicine. This complex of species consists mainly of the roots of several endangered *Anacyclus* (Asteraceae, Anthemideae). Molecular identification of these roots could increase identification accuracy, enable trade value chain monitoring, and improve the conservation of the species. However, a lack of variation in standard plastid markers for closely related species from recently diverged lineages limits the accuracy of barcoding for species identification. In this study, we developed a DNA barcoding approach using target enrichment, shotgun sequencing, and transcriptomes for accurate identification of species of *Anacyclus* based on 900 nuclear markers and complete plastome sequences. **Results:** This combined nuclear genomic and plastome dataset enables us to distinguish all species in this genus with high accuracy, as well as the geographic origin of *Anacyclus* populations across Morocco. Through the analysis of our 110 commercial samples, we show that target enrichment sequencing yields good data recovery even in processed and poorly conserved samples. **Significance:** Genomic barcoding using target enrichment sequencing overcomes many of the difficulties associated with standard DNA barcoding and is set to be an invaluable tool for the identification of medicinal plants in trade.

Harnessing the feeding habits of a ubiquitous estuarine scavenger for fish biodiversity assessment

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Background: DNA metabarcoding has rapidly become one of the most powerful tools for detecting and quantifying biodiversity in complex communities. It is also particularly useful in reconstructing the feeding habits and preferences of small organisms where morphological analysis of gut content is impracticable or unreliable. Here, we compare the diversity of fish species detected in the stomachs of brown shrimp (*Crangon crangon*) collected at multiple sites in six European estuaries, with the diversity recovered from associated sediment samples. **Results:** The employed universal COI barcode recovered a single fish species in the sediment samples and 28 species, belonging to 17 families, in the brown shrimp guts. Some of these species are common components of estuarine and coastal communities, while others represent unexpected and (or) rare taxa. **Significance:** The barcoding fragment used amplifies trace or bulk DNA template from most eukaryotic taxa, but fish and other vertebrates are generally under-represented in environmental samples. The feeding activities

of the ubiquitous brown shrimp, however, act as a sieve that high-grades for bulkier food sources, making the gut an invaluable repository of coastal fish diversity—including detection of unexpected, possibly invasive, species. We argue that metabarcoding of a widespread and abundant generalist species' gut contents may offer a more accurate and realistic picture of the biodiversity of certain taxonomic groups, at a fraction of the costs normally associated with more traditional monitoring techniques.

Insect multilocus metabarcoding: in silico evaluation of old and new primers

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Background: Many universal primers for insect metabarcoding have been reported to introduce amplification bias due to mismatches with templates. In a diverse sample, such as a soil or Malaise trap sample of insects, this may result in some species being less efficiently amplified or not detected at all. We explored the potential of solving this problem by using multiple, optimally designed, degenerate primers. **Results:** We compared published primers for insect metabarcoding with new primers resulting from the processing of all publicly available insect mitogenomes using two different primer design software packages. These primers were evaluated in silico for taxonomic coverage and resolution of corresponding individual amplicons, as well as complementarity between different pairs of amplicons. The best choice for insect metabarcoding were degenerate primer pairs amplifying fragments of the COI, 12S, and 16S genes. In addition, combining two or all of these primers significantly increases the proportion of detected and identified taxa. In addition, we propose the use of new indexes for evaluation of the quality of primers and barcodes. **Significance:** The use of degenerate primers under strict PCR conditions allows the amplification of a broad target group with a lower risk of introducing bias. Furthermore, the simultaneous use of two or more barcoding markers for the same samples facilitates the detection of taxonomic groups that just one marker would fail to detect.

Examples of discordance between morphology and DNA barcodes in lichens, fungi, and insects in Norway, NorBOL

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As a part of NorBOL, the Natural History Museum, University of Oslo, coordinates DNA barcoding of lichens, basidiomycetes, and terrestrial insects in Norway and the Arctic. Within these groups, DNA barcodes largely correspond to morphological species, but we have encountered several cases of discordance between the two types of taxonomic characters. Among fungi and lichens, we have found examples of cryptic species based on the standard barcode region for these groups, ITS. In the lichen genus *Calvitimela* (Lecanorales), there are several cryptic lineages; one example is *C. melaleuca* that was shown to consist of two cryptic species. In the gilled mushroom genus *Lepiota* (Agaricales), most morphologically described species have been revealed to consist of two genetic species. Among insects, we present an example of the reverse situation of discordance between morphology and DNA. In the parasitic wasp genus *Pteromalus* (Hymenoptera), COI and ITS2 sequences revealed two cases of several nominal species within one. Thus, although DNA barcoding to a large extent confirms conventional knowledge about species borders based on morphology, it also pinpoints taxonomic problems in need of revision.

DNA barcoding and diversity of groundwater oligochaetes in Benin (West Africa)

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Background: Groundwater is a major ecosystem in terms of biodiversity, endemism, and relict species. However, its stygofauna, the obligate groundwater fauna, remains too often ignored, although present on all continents. Its knowledge is of particular interest for public health as groundwater is also the main drinking water reservoir on earth. This study aims to build a genetic database of groundwater oligochaetes within Benin, which can be used as a reference for future studies based on DNA barcoding. It comes in a larger framework using the stygofauna as an indicator for water quality. **Results:** In total, 96 wells were sampled in 2015 and 2016, and COI barcodes were obtained from 126 specimens of the genera *Aulophorus* (Naididae) and *Haplotaxis* (Haplotaxidae), the main two oligochaete components in Beninese wells. Molecular data enabled an interesting comparison between both genera, in terms of species diversity, distribution, and dispersal capacities. The numerous specimens of the stygophile *Aulophorus* proved to consist of four species, widely distributed, with low intraspecific genetic variability, suggesting an important dispersal capacity. In contrast, seven potential species were identified in the rare stygobiotic *Haplotaxis*, each of them being restricted to one station, with one exception, so that each hydrogeographic basin can be characterized by its unique assemblage of *Haplotaxis* species. **Significance:** These first data suggest an interesting potential use of groundwater oligochaetes for water management in Benin: (*i*) the presence of *Haplotaxis* is an indicator of the phreatic origin of water in wells and, as such, suggests good water quality; (*ii*) in contrast, the presence of *Aulophorus* species in a well gives evidence of poor protection of the latter against exogenous elements, which can have a negative impact on water quality.

Phylogenetic investigation of the *Baikalodrilus* species flock (Clitellata, Naididae) endemic to Lake Baikal, Siberia

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Background: Lake Baikal is the deepest and most voluminous lake in the world, with a unique environment (ultraoligotrophic and well-oxygenated waters at all depths), and it is located in a region that is experiencing rapid climate change. It is populated with an endemic genus of oligochaetes (*Baikalodrilus*), which currently comprises 21 morphospecies. However, the validity of many species is questionable; the great similarity in their description and the lack of unequivocal diagnostic characters often lead to inconclusive species identification. In order to clarify the systematics of this genus, we analysed one nuclear (ITS) and two mitochondrial (COI and 16S) markers of 33 specimens of *Baikalodrilus* and four specimens of the genera *Spirosperma*, *Embolocephalus*, *Rhyacodrilus*, and *Haber* as an outgroup. **Results:** Phylogenetic inferences based on parsimony, maximum likelihood, and Bayesian analyses showed an early separation between two groups of species that belong to two distinct size classes and helped to re-evaluate the validity of some morphological characters as specific diagnostic characters. Three species identified prior to molecular analyses were consistent with clustering based on DNA sequences. A fourth morphospecies

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proved to be actually an assemblage of two distinct species. It was also possible to isolate a group of specimens that could be considered as a new species. Other clusters remained ambiguous, not only in terms of molecular clustering but also of morphological distinctness. **Significance:** These results will be useful for a taxonomical revision of the genus and a better assessment of the oligochaete species diversity in a lake environment facing contemporary climatic changes.

DNA barcoding establishes hidden diversity of freshwater prawns and shrimps from Neyyar River, Western Ghats, India

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Background: Western Ghats and the North-eastern Himalayan region harbors rich endemic diversity. Neyyar River, originating from Western Ghats, has an assemblage of several endemic fin and shellfishes. Due to anthropogenic factors and climate change, the pattern of river biodiversity could change over time. Baseline or reference data on endemic fauna would be useful to formulate effective management and conservation measures. The taxonomy of prawns is so ambiguous that several morphotypes have been reported as different species. **Results:** In the present study, DNA barcodes were generated for 10 species/subspecies of the genus *Macrobrachium* (*M. abrahami*, *M. aemulum keralauni*, *M. indicum*, *M. scabriculum*, *M. prabhakarani*, *M. dolicodactylus*, *M. dubius*, *M. lamarrei lamarrei*, *M. idella idella*, *M. idella georgii*) and three species of the genus *Caridina* (*C. mathiassi*, *C. gracilirostris*, *C. natarajani*). The average confamilial, congeneric, and conspecific distance values were 16.68%, 12.24%, and 0.446%, respectively. The studies revealed that *M. dubius*, *M. abrahami*, *M. prabhakarani*, *M. dolicodactylus*, and *M. scabriculum* exhibited similar barcode data and low K2P divergence (0.4%) between these specimens, giving sufficient reason for synonymizing them. High genetic divergence (19.5%) was observed between *M. amelum keralauni* and *M. amelum*. This supports possible elevation of the subspecies *M. amelum keralauni* to species level, *M. keralauni*. DNA barcodes for *M. indicum* and *C. mathiassi* are generated for the first time, and the presence of these species is confirmed in Neyyar River, Kerala State. **Significance:** The present study paved the way to confirm the status of prawns and shrimps of Neyyar River. Apart from establishing species diversity, the present study helped to discover new species, generate new barcode data for species of the river, and focus on endemism. These could be useful tools for resource management and conservation.

From TOPS to bottom: using DNA barcoding to combat the illegal harvest of threatened plant species in South Africa as recorded in the NEM:BA TOPS list 2015

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Background: Overexploitation of natural resources is a major threat to biodiversity as it may lead to the extinction of rare and vulnerable species. According to the *National Biodiversity Act* (NEM: BA) act 10 of 2004: any person who is in possession of a species that is listed as protected or threatened or owns any product derived from these species must have a valid permit to do so. Countries with unique floras, such as South Africa, face major challenges in protecting indigenous plants from specialist collectors while having to manage other threats such as habitat destruction, biological invasions, and climate change. DNA barcoding can be used as a tool in aiding plant conservation. In this study, core DNA barcoding regions (*rbcLa* and *matK*) were used to assist in identifying the threatened plant species of South Africa. **Results:** Based on genetic divergence, PCR amplification efficiency, and the BLAST algorithm, the core DNA barcodes proved to be efficient. Hence a DNA barcode library was created on the Barcode of Life Data System (BOLD) to assist in future identification of unknown or taxonomically doubtful species. **Significance:** Plant diversity is not only crucial in maintaining healthy and sustainable ecosystems, but it also holds socio-economic value. In order to implement strategies to protect threatened species, identification of these species is a critical first step.

The effects of ecological traits on the rate of molecular evolution in bony fish: a multivariate approach

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Background: A myriad of environmental and biological traits have been investigated for their roles in influencing the rate of molecular evolution. However, most studies have focused on a single ecological trait, while controlling for additional factors in an informal way, generally by excluding taxa. The public availability of DNA barcode sequence data, in conjunction with online trait databases, provides the opportunity to perform a more comprehensive study that considers the effects of multiple traits at a broader phylogenetic scale and in a multivariate context. **Results:** This study utilized a dataset comprised of cytochrome *c* oxidase subunit I (COI) barcode sequences from over 6000 bony fish species to investigate the effects of 30 biological and environmental traits on molecular evolutionary rates. Latitude and environmental temperature were included, which have been previously implicated as correlates of mutation rates in fish, as were traits related to population structure and habitat. Additional traits previously associated with metabolic rate differences in fish, such as morphology, locomotion, and feeding habits, were also investigated. A bioinformatics pipeline was constructed to manipulate and assemble both DNA barcode data retrieved from the Barcode of Life Data System (BOLD) API and trait data obtained from FishBase. Results from our multivariate analysis, accounting for phylogeny, revealed overall substitution rates to correlate most significantly with age at maturity and longevity. **Significance:** These initial results provide evidence for the importance of life history rate correlates in bony fish relative to other traits, while also showcasing the efficiency of using bioinformatics tools to assemble and analyze biological information obtained from different online databases. The bioinformatics pipeline (publicly available through https://github.com/jmay29/phylo) may be easily adapted to investigate additional organismal groups and molecular rate correlates, thereby providing a solid and amendable foundation for future work in this field.

Phylogeographic investigation of indigenous and invasive *Tamarix* (saltcedar) based on nuclear ITS, plastid *trnS-trnG*, and microsatellite DNA markers

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Background: Three species of the saltcedar (*Tamarix*: Tamaricaceae) occur in South Africa, although only *T. usneoides* is indigenous and is effectively used in southern Africa mines for phytoremediation. *Tamarix ramosissima* and *T. chinensis* were reportedly introduced into South Africa from Eurasia also for phytoremediation, but they have since invaded riparian zones. Species of *Tamarix* are morphologically similar, and hybridization adds to their taxonomic confusion. This investigation aimed to identify populations of pure *T. usneoides* for phytoremediation use and to reveal the geographic origin of the invasive species to facilitate a biocontrol programme. Nuclear (ITS) and plastid (*trnS-trnG*) DNA sequence data and microsatellites (co-

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dominant markers) were used to characterize species of *Tamarix* and their hybrids. **Results:** Phylogenetic analyses based on the sequence data separated the indigenous from the exotic species of *Tamarix*. However, the ITS and *trnS-trnG* sequences were not informative enough to distinguish the exotic species and to infer hybridization events. Population genetics structure using microsatellite markers confirmed the presence of three species and their hybrids and revealed that the *Tamarix* infestation in South Africa is dominated by hybrids between the two exotic species (>65%). The indigenous *T. usneoides* showed a strong genetic differentiation (F_{st}=0.197) as compared to the exotic species (F_{st}=0.139), with the hybrids showing the lowest differentiation (F_{st}=0.048). **Significance:** This study represents the first time genetic markers are used to clarify the identity of *Tamarix*, a taxonomically difficult genus, in South Africa, and identifies populations of pure *T. usneoides* to be propagated for phytoremediation. It also reveals the *Tamarix* genotypes that should be included in host-specific trials of a potential biocontrol agent for the control of invasive genotypes. The strong genetic differentiation between the indigenous and the invasive species should be encouraging news from a biocontrol point of view.

DNA barcodes library for the Kenya endemic woody plants

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Background: The flora of Tropical East Africa is rich, with about 12 100 described species of vascular plants. This diversity is found in diverse ecosystems, ranging from forests, wooded grasslands, and Afrotemperate highlands. Among the native (and a small component of naturalised) species of East Africa, about 2500 species are endemic to the region, and 58% are recorded in Kenya. About 1800 vascular plant species in Kenya are woody plant taxa, of which 50 species are endemic to the country. Despite the above wealth of vascular plants, the available identification tools are limited to taxonomic (dichotomous) keys, but such keys require specimens that have full vegetative and reproductive characters. Given that a number of Kenyan plant species are in trade, locally and internationally, there is an urgent need for additional identification tools suited for fragmented plant material such as herbal preparations. DNA barcodes offer a rapid tool for the identification of diverse material. The Barcode of Life repository (http://www.boldsystems.org/) is exponentially growing, with about 5 360 000 specimens with barcodes from over 264 000 species worldwide, and nearly 63 000 flowering plants species included. **Results:** Here, we present results on a recently started effort to establish a DNA barcode library on the Kenyan flora. **Significance:** While our initial focus is on the endemics, we anticipate to expand to other representative lineages, and to use these data towards studies on evolution, use, and conservation.

DNA metabarcoding reveals differences in plant-associated soil micro- and macrobiomes across bacteria, fungi, and invertebrates

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Background: Simultaneous assessment of above and below-ground biotic components are of particular interest as individual tree species are likely to have a direct effect on the composition of surrounding soil biotic communities. Prior research demonstrated individual tree species influence on soil microbial community composition and nutrients for temperate tree species, but less for tropical species. Determining the importance of plant-species effects on below-ground biotic communities in tropical forests is essential for predicting current and future biodiversity. This is particularly important as both deforestation and drought are expected to intensify over the next century. However, considerable effort is needed to include and study the interactions of soil invertebrates with individual plant species and microbes. 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 ammonium, nitrate, 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 ammonium 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. 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. These findings provide an avenue via DNA metabarcoding for future assessment of conservation efforts that facilitate plant-species reintroduction programs.

A first, local DNA barcode reference database of the forensically important flies (Diptera) of the island of La Reunion

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Background: Forensic entomologists use fly larvae of the order Diptera to establish the time interval between death and body discovery. The identification of these flies is decisive in forensic casework but is hampered by difficulties in identification and the potential presence of fly larvae that are of no forensic interest. The identification of forensically relevant fly species, and their discrimination from non-forensically important species is facilitated with DNA barcoding but only if a representative local reference barcode library is available. **Results:** We constructed a local reference library of 195 COI barcodes from 29 species of the families Calliphoridae, Fannidae, Muscidae, and Sarcophagidae from the island of La Reunion. Our results show that (*i*) the library contains most of the forensically relevant species of these families from the island, and (*ii*) all fly species can be unambiguously identified with DNA barcoding using a variety of analytical methods. Two public libraries (GenBank and the Barcode of Life Data System (BOLD)) only allowed to identify half the number of species of these families present in La Reunion, showing that both libraries are not representative for this island fauna. Furthermore, 9 of 10 species with a forensic interest could be identified using both public libraries, showing that, for forensic casework, the libraries prove helpful. **Significance:** This is the first DNA barcode reference database for the forensically important fly species of La Reunion. The database will contribute to the growing use of dipteran larval composition on corpses to estimate the post-mortem interval.

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Solving crimes: a forensic rove beetles (Staphylinidae) barcode database for Belgium

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Background: Rove beetles (Coleoptera: Staphylinidae) are early-stage visitors on corpses. They deposit their eggs on the corpse and the emerging larvae feed on fly larvae. The duration of development from egg to adult is species specific and can be used in forensics to estimate the post-mortem interval (PMI), i.e., the time elapsed between death and moment of discovery of a corpse. In addition, the identification of rove beetle larvae can provide information about a potential displacement of the corpse or the manner and cause of death. Therefore, correct identification of rove beetle life stages is important for crime investigators. Fortunately, the species identification can be enhanced by using DNA barcodes, provided that a reliable reference library is available. At present, the forensically important species of western Europe are not, or only poorly, represented in public databases (e.g., GenBank, BOLD). In order to remediate this gap, the presented project aims at constructing a reference library for 60 rove beetles species found in Belgium. **Results:** Morphologically identified voucher specimens (currently some 200 specimens comprising 48 species) were obtained from several research institutes in Belgium. In addition, fresh samples were obtained from pig cadavers. Due to the age and preservation of the voucher specimens, the extracted DNA was fragmented, and PCR protocols were optimized using previously described internal primers to amplify smaller overlapping amplicons (388 and 403 bp long). These short sequences were aligned and assembled into a composite sequence in order to generate full-length barcodes per specimen. **Significance:** Compiling the barcode reference library and optimizing the laboratory protocols will allow forensic investigators to quickly and accurately identify rove beetles at all life stages found on corpses.

Shotgun sequencing plant DNA: selection of material and methods

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Background: In total about 3000 plant samples of the Norwegian and Arctic flora have been barcoded from the collections at Tromsø University Museum, northern Norway, through the Norwegian Barcode of Life (NorBOL). Initially, 1805 specimens of 564 species were sequenced for *rbcLa* and ITS2 using Sanger sequencing. As these standard barcodes have limited taxonomic resolution, we developed a protocol for extraction of high-quality DNA using NucleoSpin 96 plant II kit (Macherey-Nagel), followed by shotgun sequencing to obtain the full plastid, mtDNA, and ribosomal DNA. **Results:** Our Sanger analyses had a success rate of 85% for *rbcLa*. For ITS2, sequences for 74% of the specimens were obtained, but all were too short (<500 bp) to gain barcode status. The overall success rate decreased with later collection date (May to August) from 92% to 76%. Based on this knowledge, samples for the plastid and ribosomal genome sequencing were selected, and DNA was extracted from all samples, representing 97 plant families. Preliminary results of the shotgun sequencing based on auto assembly of 285 specimens gives a success rate of 82% and 72% for *rbcL* and *matK*, respectively. *Carex* (25 specimens) was excluded from the preliminary results for *matK*. For ITS2, sequences were obtained for 67% of the specimens, but all were less than 500 bp. **Significance:** Herbarium specimens have been an excellent source for building up both the standard barcode and the low-coverage full genome reference library. These libraries will be publically available. Building up a library consisting of not only a few barcodes but also the plastid and the ribosomal genomes is beneficial for the international research community as it enables researchers to address more complex topics.

Establishment of a quality-controlled secondary fungal barcode (TEF1α) database for medical fungi

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Background: Correct and fast identification of the causative 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. The current primary fungal DNA barcode is the internal transcribed spacer (ITS) region. In 2015, an international consortium of medical mycology laboratories established the ISHAM-ITS database, the first quality-controlled fungal barcode database for human and animal pathogenic fungi. Clinically important species show a low intraspecies variability and a clear barcoding gap at the interspecies level; consequently, ITS sequencing can be reliably used for the identification of most species. However, for some species an alternative barcode locus needs to be introduced to ensure reliable identification. **Results:** A recent study identified a number of possible new loci and tested them on a broad taxonomic range of fungi to ensure an accurate and reliable species identification in a clinical setting. Based on the general requirements of a barcode, such as amplification efficiency under standardized laboratory conditions, and the universality of the primers across different taxa, the translational elongation factor 1α (TEF1α) was proposed as an official secondary barcode. However, there is currently no dedicated quality-controlled database for the secondary barcode. The aim of the current research is to generate TEF1α sequences for medically relevant species to complement the ISHAM-ITS database and to establish a new reference database for TEF1α. The intra- and interspecies variations of TEF1α locus compared to that of ITS region were evaluated. The TEF1α shows less intraspecies and higher discriminatory power at the interspecies level than the ITS, and TEF1α improved the barcoding gap in some taxa. **Significance:** The application of a dual DNA barcoding system enables all clinically important fungal pathogen to be accurately identified.

Searching for hidden diversity among the phylum Platyhelminthes using global metabarcoding data

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Background: During the last decade, several initiatives have been taken to explore the marine diversity using high-throughput technologies. As a result, a bulk of metabarcoding data are now available. Using these data, we aimed to better understand the real diversity of the acoelomate Platyhelminthes, an evolutionary and ecologically important clade of animals with both free-living and parasitic species. **Results:** In this study, we analyzed data from seven marine (Malaspina, METABARPARKS, TaraOcean, TaraArctic, BioMarks, Blanes, DeepSea) and two freshwater (Paraná River, Lake Sanabria) metabarcoding projects under a phylogenetic framework. Our main objective is to get a better insight into the diversity of Platyhelminthes and to recognize novel clades of the free-living representatives of this phylum. To achieve our goal, we first built a reference tree using 18S rDNA GenBank sequences covering all known phylogenetic diversity of Platyhel-

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minthes. We performed a phylogenetic placement of the clustered operational taxonomic units (OTUs) from the different environmental datasets in order to retain sequences with interesting phylogenetic position within, or close to, Platyhelminthes. We then used these sequences to reconstruct the phylogeny of the phylum. Furthermore, we combined our phylogenetic results with data of abundance and other ecological parameters to assess the worldwide distribution of the different clades within Platyhelminthes. **Significance:** To our knowledge, this is the first effort to compile such a rich and diverse dataset in order to address the question of hidden diversity within the phylum Platyhelminthes. Apart from the well-studied planarians, polyclads, and neodermatans, very little is known about the rest of the flatworm orders that are usually collectively referred to as "microturbellarians". We expect not only to better understand the diversity patterns of all Platyhelminthes but to also unravel previously undescribed free-living clades, to gain a better understanding of the microturbelarian "dark matter" and to make inferences of their ecology.

DNA barcoding for identification of small-sized beetles from steppe areas of the Republic of Moldova

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Background: Steppes in the Republic of Moldova are part of the unique Eurasian steppe ecosystem, supporting a rich flora and fauna, and providing invaluable ecosystem services. Steppes are little involved in the network of protected areas, and their degradation continues. Insect communities of the steppes are poorly known. Particularly, identification of small-sized beetles represents a major challenge. For many taxonomic groups, DNA barcoding proved to be a useful, standardized tool for species identification. **Results:** This study is the first attempt to reveal the small-sized beetle diversity of the steppe areas of the Republic of Moldova using the DNA barcoding tool for identification. DNA was extracted from 95 specimens and depending on the size of the sampled individual, one or two legs were used. Voucher specimens are deposited in the collection of the Entomological Museum, Institute of Zoology of the Academy of Sciences of Moldova. COI sequences were obtained from 77 specimens, of which 61 (79%) were barcode compliant. Based on the Barcode of Life Data System (BOLD), 39 (51%) specimens were identified at species level (18 species), 34 (44%) at genus level (15 genera), and 4 (1%) were not assigned to a lower-level taxon. **Significance:** The current study indicates that the DNA barcoding tool is effective for identifying species of small-sized beetles and facilitates the discovery of new beetle species. Obtained sequences will contribute to extending the reference library of Coleoptera. The availability of barcode data will help to solve taxonomic confusion and reduce the time needed for species identification.

Construction of a baseline for zooplankton from the biggest karstic sinkhole in the south of Yucatan Peninsula (Mexico)

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Background: Even though DNA barcoding has become extremely popular since Hebert's proposal in 2003, until now, there has not been a single baseline created for zooplankton in any freshwater system in the world. This is due to the fact that zooplankton species are often hard to barcode and that sampling methods have not changed in the past 100 years. In this study, we established a baseline of the Cenote Azul using new sampling methods and one single set of primers (Zplank) for all groups collected. **Results:** The Cenote Azul is a karstic, oligotrophic system that is 74 m deep and 200 m in diameter without a littoral zone. We used a combination of plankton nets (50 and 300 μm mesh size) and light traps of our own design for collections. We registered a total of 40 taxa including 4 cladocerans, 4 copepods, 2 ostracods, 1 palaemonid, 2 fish larvae, 2 rotifers, 1 isopod, 1 bivalve, 7 chironomids, and 13 arachnids. We believe that this number will increase with additional sampling. At least half of all the species that we found have not been registered in the Barcode of Life Data System (BOLD) or in previous species lists from this location. There was a significant difference in the number of taxa collected with the plankton nets and with the light traps. Only eight taxa were collected in the nets, while almost all the taxa recorded were present in the light traps. **Significance:** The results of this study demonstrate that light traps were an effective method for a rapid evaluation of zooplankton in this system. Combining DNA barcoding and next-generation methods will enable us to perform rapid evaluations to determine the conservation status of these aquatic systems. For this reason, we believe that it is fundamental to first elaborate species baselines in these ecosystems.

Moina macrocopa: another complex of species in a common Cladocera

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Background: The genus *Moina* encompasses many complexes of sibling species with worldwide distributions as in the case of *Moina micrura*, *Moina brachiata*, and the target of this study, *Moina macrocopa*. Many of these species have been described in Europe during the 19th century, and the material used for these descriptions is lost. Most of the keys for identification all over the world are based on a single species, usually European in origin, leading to large confusion as to their real identity. **Results:** Molecular analyses based on COI demonstrate that *M. macrocopa* is a complex of at least two species. Moreover, we found *M. macrocopa macrocopa*, the European species, in a temporal pond in Calderitas, México, living in sympatry with some congeners of the *M. micrura* complex. The COI gene shows a mean divergence of 7.04% between the American *M. macrocopa americana* and the European *M. macrocopa macrocopa* sequences. Morphological analyses and molecular results indicate that they are clearly distinct species. **Significance:** The significance of this study is to reorder one of the most common cladocerans in the world, belonging to the complex *M. macrocopa* and demonstrates that it is possible to distinguish them. This study reaffirms that an integrative taxonomical approach is necessary and useful to delimit species.

Key limitations to aquatic eDNA metabarcoding: a cautionary case study from a diverse public aquarium

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Background: Environmental DNA (eDNA) and DNA metabarcoding techniques have been widely touted as powerful new tools for monitoring biodiversity in aquatic ecosystems. However, these techniques are still in their infancy and require thorough validation because there are still several key uncertainties surrounding eDNA metabarcoding. These uncertainties include both methodological and analytical limitations that must be addressed before there is wider adoption of eDNA metabarcoding in biodiversity monitoring. In this study, we assess both the ability for eDNA metabarcoding to capture biodiversity in a highly diverse closed system at the Ripley's Aquarium of Canada in Toronto, Ontario, as well as address a number of knowledge gaps pertaining to eDNA metabarcoding to open a discussion on current issues limiting this tool. **Results:** This study found that eDNA metabarcoding recovered 62 of 107 (58%) target species and 30 of 44 (68%) target genera from a closed system when using a multimarker (COI, 16S, 12S) approach. Additionally, individual markers showed great disparity in off-target identification noise, with COI pro-

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ducing the greatest proportion of noise (95% of operational taxonomic units (OTUs)). **Significance:** This case study represents the first to highlight key uncertainties and current challenges for eDNA metabarcoding as a biodiversity monitoring tool in highly diverse closed aquatic ecosystems. We identify several outstanding issues with eDNA metabarcoding relating to contamination, sampling methodology, study design, statistical and bioinformatic analyses, and a lack of standardized protocols. These issues raise concerns for the reliability of eDNA metabarcoding when applied to studying complex and highly diverse natural systems. These concerns are reminiscent of those identified previously for DNA barcoding and ancient DNA work. We conclude that the key facets of eDNA metabarcoding methodology that we identify here require further focus before eDNA metabarcoding can be broadly applied in aquatic biodiversity monitoring.

Metataxonomic analysis of microbial community changes in *Fusarium* wilt-infected banana crops from Colombia

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Background: The fungus *Fusarium oxysporum* f. sp. cubense (FOC) is one of the most destructive wilt-causing diseases affecting bananas worldwide. Our aim was to compare and evaluate microbial communities in soils and roots of Gros Michel banana cultivar plants with and without the disease. Bacterial 16S rRNA gene and fungal ITS metabarcoding were used to study the microbial community composition of 26 soils and 15 roots from banana crops. **Results:** 16S rRNA analysis showed that Acidobacter, Proteobacteria, Verrucomicrobia, Actinobacteria, Chloroflexi, Nitrospirae, Firmicutes, Bacteroidetes, Planctomycetes, and Gemmatimonadetes were the most abundant phyla in soils and roots, comprising 58.3% of all read sequences. Comparative microbiome analyses performed between healthy and diseased banana plants in *Fusarium* wilt-infested fields in Colombia revealed significant shifts in Chloroflexi, Firmicutes, and Bacteroidetes, which were the most abundant in healthy plants, while Nitrospirae was more abundant in diseased plants. ITS analysis showed Ascomycota, Basidiomycota, Glomeromycota, and Zygomycota to be the most abundant phyla in soils and roots, comprising 9.44% of all read sequences. Moreover, we used PICRUSt to predict the functional composition of each microbial community metagenome based on its 16S profile. When healthy and diseased soil samples were compared, the results showed a decrease of the subcategories carbohydrate, amino acid, and energy metabolism pathways, membrane transport, and replication and repair, respectively. In contrast, the comparison between healthy and diseased root samples showed an increase of the subcategories membrane transport, energy, carbohydrate, amino acid, cofactors, and vitamins metabolism pathways, in addition to replication and repair. **Significance:** This is the first effort to specifically identify antagonistic and synergistic relationships between microorganisms associated with *Fusarium* wilt disease in Colombia, in order to propose strategies for disease prevention and management. Therefore, metagenomics can represent an important approach for determining crop health.

Taxonomy, phylogeography, and evolution of marine hydroids of the superfamily Plumularioidea (Cnidaria, Hydrozoa)

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Background: Marine hydroids are common in shallow and deep waters worldwide, with many species presumably presenting ample distributions. Their taxonomy is controversial, as only few diagnostic morphological characters are available to categorize taxa. Their genetic relationships are also little investigated. **Results:** We aim to understand taxonomic, phylogeographic, and evolutionary relationships within the superfamily Plumularioidea. Integrating a classical taxonomic approach with the DNA barcoding of 678 individuals, we accessed phylogenetic relationships between 678 16S genotypes of more than 200 species. **Significance:** We uncovered significant new and cryptic diversity at the levels of species and genera, but also few cases of synonymies. The few species recognized to effectively present wide geographical distributions either exhibit large population sizes, release medusoids, and (or) raft with boats. Thermal tolerance, oceanic currents, and land barriers were revealed as important drivers for dispersal and speciation. The geographical origin and diversification timings of Caribbean, eastern Pacific, and eastern Atlantic hydroids were hypothesized in relation to past climatic and oceanographic marked changes.

The fungal phytobiome of *Searsia lancea* (karee) trees with Karee Malformation Disease in South Africa

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Background: When looking at the plant microbiome, the fungal biota (mycobiome) constitutes an important component. These fungi occur on the inside (endophytes) or the outside (epiphytes), play various roles, and differ in community structure based on host, substrate, and tissue type. Novel approaches that are culture-independent can be employed to investigate this diversity, such as next-generation sequencing methods. *Searsia lancea* (karee) is a common native tree in South Africa. A new disease called Karee Malformation Disease (KMD) consists of malformations occurring mostly on vegetative and floral tissues. The aim of this study was to use a metagenetic approach to characterize the mycobiomes found in the floral and vegetative tissues of *S. lancea* to determine if there are differences between communities in malformed and healthy tissues. A baseline of how a typical fungal phytobiome of karee would be structured was also established for more accurate comparisons with diseased tissues. Mini-barcodes using the ITS regions of the ribosomal operon, and the Translation Elongation Factor 1-α gene regions, were generated with Illumina sequencing and analysed in a bioinformatics pipeline. **Results:** As expected from past literature, community differences were observed in a normal tree between different tissue types, and between young and old tissues. Malformed tissues differed greatly from their healthy counterpart tissues in the fungi infecting them. Potential secondary fungal pathogens were observed in older and dying malformations. **Significance:** No dominant fungal group that could be the cause of the disease was detected, thus confirming previous studies concluding that fungi most likely are not the cause of the disease. However, it is clear that the malformations greatly changed the fungal communities that would normally be present in an unaffected tree and present a niche of its own within the tree.

A genomic insight on the species boundaries in *Hyles euphorbiae* group of hawkmoths: are DNA barcodes failing in telling good species apart?

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Background: The hawkmoth genus *Hyles* Hübner, 1819 (Lepidoptera: Sphingidae) comprises 32 species globally. Their taxonomy has proven to be difficult to elucidate because of wide distributions of

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many species, morphological geographic variability, small genital differences, and poor resolution of mt DNA markers, including DNA barcodes. We studied the taxonomy of the five species of *Hyles euphorbiae* complex (HEC) based on genome-wide ddRAD sequencing data. **Results:** In total, 95 analyzed specimens of *Hyles* yielded ddRAD data of 1 401 769 base pairs, 7286 loci, and 94 126 SNPs. Out of 95 specimens, 60 represented the HEC complex. STRUCTURE analysis did not reveal clear distinction between any of the five species. Similarly, the SNAPP species tree and BDF* delimitation did not support the status of the HEC species as separate entities. The TreeMix analysis revealed significant levels of gene flow between the species have taken place. **Significance:** Based on a genomic data of over a million of base pairs and thousands of loci, the species boundaries in HEC group are poorly defined, demonstrating that the failure of DNA barcodes to separate species is largely due to the operational factors, i.e., oversplitting of species. The species in the HEC complex represent local or geographical forms with frequent gene flow between them, and they should not be ranked as separate entities at species level. We anticipate similar situations in many other taxonomically complex groups, especially where species show strong geographic patterns.

Disentangling phylogenetic patterns of the mistletoes (Santalales) occurrence in sub-Saharan Africa

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Mistletoes, aerial hemiparasitic flowering plants in the superasterid clade, arouse wide curiosity among naturalists. Yet there are gaps in our knowledge on the occurrence of the mistletoes both geographically and in relation to their hosts in sub-Saharan Africa. Here, we use herbarium records housed in major herbaria in the continent (Pretoria, Nairobi, Natal, and Cape Town) to extract information on geographical distribution and host preference. Using a robust phylogenetic tree of southern Africa woody plants, based on the DNA barcodes, we infer host preference patterns of the mistletoes. Mistletoes are widespread in sub-Saharan Africa, occurring in most biomes and major vegetation types and across diverse elevation and climatic gradients. We noted over 150 parasite-host interactions, about two thirds involving mistletoes in Loranthaceae and the one third involving Viscaceae. The most prevalent parasites, *Erianthum dregei* and *Viscum rotundifolium*, were also widespread and occurred on multiple hosts. No incidence of parasitism was observed among gymnosperms and monocots and only a single magnoliids host (Annonaceae) was observed. Overwhelmingly, the hosts belonged to eudicots in the rosid clade and especially among the Fabales (~100 host-parasite interactions). Even within the Fabales, there is phylogenetic clustering of the interactions, especially among the mimosoids (*Vachellia*, *Senegalia*) but conspicuously absent among the papilionoid legumes. In this talk, we further disentangle age and spatial patterns of the host–parasite interactions, testing whether such interactions are associated with particular host plant traits.

DNA barcoding and wildlife forensic investigations: the South African experience

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Background: Illegal wildlife trade has continued to escalate globally, leading to significant declines in the populations of wildlife species in Africa. Forensic genetic services have therefore become vital as a conservation approach in assisting with the monitoring and prosecution of illegal trade. Illegal activities generally include possession of protected species that are confiscated by police and customs officials, wildlife poaching for local bush meat markets and traditional medicines. The number of species targeted by black markets has also continued to grow, necessitating the need for developing validated and accurate species-specific genetic technology for forensics. **Results:** This study used a reference database of 126 expertly identified priority and look-alike species of birds, mammals, and reptiles collected under chain of custody through the Barcode of Wildlife Project (BWP). All references (5/species) were sequenced using the COI and Cytb mtDNA genes. Sequence divergences indicated high levels of interspecific variation (0.2%–25%), with some geographic sub-structuring in some species. This DNA barcode database was also effectively used to identify 325 unknown crime scene samples from 61 forensics cases from the region between 2015 and 2017. The main wildlife species identified have included local protected species illegally traded in high numbers such as abalone, elephant, lion, parrots, and pangolin. Other economically important local species identified from poaching incidences include several antelope species (duiker, kudu, and waterbuck). This database was also successfully applied in identifying unknown pangolin scales from 10 confiscations in Asia originating from Africa. **Significance:** The DNA barcoding technology has become a valuable genetic tool for species identification for wildlife crime prosecution in South Africa. There is, however, a need to improve this database to cover species ranges to ensure that geographic identification (where possible) can trace sources and illegal trade routes. Some examples of the challenges encountered by the project will be discussed.

SEAKEYS: unlocking foundational marine biodiversity knowledge in South Africa using DNA barcoding

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Background: The information that is available on marine biodiversity in South Africa is limited for most ecosystems. The development of comprehensive databases is therefore critical to support the effective monitoring of marine ecosystems and the implementation of evidence-based policy and conservation management for sustainable use. The SEAKEYS project, funded by the DST/NRF/SANBI Foundational Biodiversity Information Programme (FBIP), therefore aims to generate, manage, and disseminate fundamental information on South African biodiversity using DNA barcoding to establish species databases and distribution records for priority ecosystems and groups. **Results:** DNA barcodes of 573 specimens of coastal marine fishes and invertebrates from 128 nominal species were analysed from the cool-temperate, warm-temperate, and sub-tropical bioregions, and the offshore Agulhas Bank in South Africa, and Inhaca Island in southern Mozambique. These specimens included invasive, commercially important, rare, threatened, and endangered species. The average COI sequence divergences within species, genera, and families were moderate to high at 1.76%, 20.0%, and 23.5%, respectively. The barcode-gap analysis indicated that 14 species were indistinct, while 23 had high intraspecific distances (>2%) due to either deep divergences or cryptic speciation. An additional 12 species had very low interspecific variation suggesting misidentifications or a need for taxonomic evaluation. The 138 Barcode Index Numbers (BINs) identified included 93 concordant assignments and 17 singleton specimens that will require more sampling for species verification. Discordant BINS (28) with good sample sizes (5–65 samples) were re-analysed and corrected. **Significance:** These DNA barcoding analyses suggest that the diversity of marine fauna in South Africa, a known area of endemism, has been underes-

timated. The availability of vouchered specimens from this study will be critical in linking all new and unclear BIN clusters to species and new descriptions. Therefore, this SEAKEYS DNA barcoding project will contribute towards the estimation of marine biodiversity in South Africa.

Barcode of wildlife project Kenya: role of biorepositories and DNA barcode reference library in wildlife crime prosecution

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Background: Kenya is a partner country in the Barcode of Wildlife Project (BWP). The Barcode of Wildlife Project Kenya (BWPK) aimed to construct a barcode reference library of endangered flora and fauna for law enforcement in wildlife crime, with National Museums of Kenya (NMK) and the Kenya Wildlife Service (KWS) as lead institutions. NMK is a repository of live and voucher plant and animal specimens, while KWS is the enforcement and management agency. BWPK selected 200 priority species of national importance and conservation concern (frequently encountered in court cases and protected by law), CITES-listed and look-alike species. Specimens were sourced from existing repositories, botanic gardens, private collections, and from nature. **Results:** Training in DNA barcoding chain analysis was undertaken; Standard operating procedures (SOPs) for field collection and laboratory analysis were developed. Over 1000 "barcodes" have been generated and submitted to GenBank. Plant voucher specimens for these barcodes are deposited in East Africa Herbarium and NMK Botanic Garden. Animal e-vouchers and vouchers for small mammals, fish, reptiles, invertebrates, birds and amphibians are deposited in respective collections at NMK. These voucher specimens can be revisited in case of doubt on viability of reference library. They also serve as reference points for identification of exhibits. Sub-sampled tissues and genomic DNA are maintained at NMK. **Significance:** Identification of wildlife exhibits and verification based on the created barcode reference library were successful. The expert evidence generated is being used in Kenyan courts for wildlife crime prosecution, and two convictions have been secured. More exhibits are undergoing identification and this will lead to more convictions. Increasing sequences on Kenyan wildlife is vital in the development of a reference library of species in wildlife crime, with enhanced capacity to use DNA-based methods to combat wildlife crime in Kenya.

Developing DNA barcode reference library for aphid species in Pakistan

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Background: Aphids (Hemiptera: Aphididae) are important crop pests and disease vectors, but their correct identification to species is a challenge. Sequence variation in the mitochondrial cytochrome *c* oxidase I (COI-5′) (DNA barcode) gene has proved effective for the identification of insect species. The success of DNA barcoding for species identification relies on the availability of reference barcode libraries, which are lacking for the aphid fauna of Pakistan. **Results:** This study employed morphology to identify 809 aphids collected in Pakistan and analyzed their DNA barcodes to validate species identification and develop a reference library. The sequences were submitted to the Barcode of Life Data System (BOLD), where they were assigned to Barcode Index Numbers (BINs), species proxies. Morphology identified 743 aphids to 40 species, while the other 66 could be assigned only to the genus or the family (Aphididae). Three species (*Periphyllus lyropictus, Aphis nasturtii, Aphis astragalina*) were found for the first time in Pakistan. The BIN system assigned the 809 sequences to 52 BINs, that were supported by neighbour-joining analysis and Bayesian inference. Conspecific barcode distances ranged from 0% to 10.2% (mean=0.2%), while congeneric ranged from 0.4% to 10.3% (mean=7.3%). Analysis suggested that specimens with >3.0% intraspecific divergence actually involved a species complex. In fact, sequences for three major pests (*Aphis gossypii*, *Sitobion avenae*, *and Aphis craccivora*) showed deep intraspecific divergences, pointing towards existence of cryptic species complexes. Haplotype analysis including global barcode data for seven virus-vector aphid species (*Acyrthosiphon pisum*, *Aphis spiraecola*, *A. gossypii*, *Myzus persicae*, *Rhopalosiphum maidis*, *Rhopalosiphum padi*, *S. avenae*) showed a significant genetic variation among regional populations. **Significance:** The study compiles the first DNA barcode reference library for the aphids of Pakistan, providing means for sequence-based species identification of regional aphid fauna. Haplotype analysis of virus-vector species at a global scale has significance for understanding disease spread by aphid genotypes.

Resolving taxonomic ambiguity and cryptic speciation of species of *Hypotrigona* through morphometrics and DNA barcoding

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Background: Stingless bees are important pollinators of cultivated and wild plants, contributing significantly to biodiversity and food security. Understanding pollinator–plant interactions is essential to secure these ecosystems services. The use of morphological features in the identification of stingless bees in the genus *Hypotrigona* is extremely difficult due to many similarities among species resulting in taxonomic ambiguity. Here, we apply both traditional morphometrics and DNA barcoding as complementary tools for the identification of three species of *Hypotrigona*: *Hypotrigona gribodoi*, *H. ruspolii*, and *H. araujoi*. **Results:** Our results show that morphometrics separates *H. gribodoi* and *H. ruspolii* from *H. araujoi*; however, there is an overlap between *H. gribodoi* and *H. ruspolii*. On the other hand, DNA barcoding separates the three species reliably and consistently. However, there is lower genetic divergence between *H. araujoi* and *H. gribodoi* from Kakamega (1.4%) than between *H. gribodoi* collected from Kakamega and *H. gribodoi* from Mwingi (4.3%). The low genetic distance between *H. araujoi* and *H. gribodoi* suggests hybridization, while the high intraspecific distance in *H. gribodoi* strongly suggests cryptic speciation within this species. **Significance:** The combined use of morphometrics and molecular taxonomic approaches (DNA barcoding) provides a convenient, robust, and reliable way to identify species of *Hypotrigona*. The high intraspecific divergence highlights the need for a thorough revision of *H. gribodoi*.

Identification of stingless bees (Hymenoptera: Apidae) in Kenya using morphometrics and DNA barcoding

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Background: Stingless bees are important pollinators of wild plants and crops. The identity of stingless bee species in Africa has not been fully documented. The present study explored the utility of morpho-

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metrics and DNA barcoding to identify African stingless bees and to further employ these tools to identify potential cryptic species. Stingless bee samples were collected from three ecological zones, namely Kakamega forest, Mwingi forest, and Arabuko-Sokoke Forest, which are geographically distant and cover high, medium, and low altitudes, respectively. Forewing and hind leg morphometric characters were measured to determine the extent of morphological variation between the populations. DNA barcodes were generated from the mitochondrial cytochrome *c* oxidase I (COI) gene. **Results:** Principal Component Analysis (PCA) on the morphometric measurements separated the bees into three clusters: (1) *Meliponula bocandei*, (2) *Meliponula lendliana* + *Plebeina hildebrandti*, and (3) *Dactylurina schmidti* + *Meliponula ferruginea* black + *Meliponula ferruginea* reddish brown. But Canonical Variate Analysis (CVA) separated all the species, into individual clusters, barring two morphospecies (*M. ferruginea* reddish brown, *M. ferruginea* black). The analysis of the COI sequences showed that DNA barcoding discriminated all the species included in this study, and it revealed remarkable genetic distance (7.3%) between the two morphs of *M. ferruginea*. **Significance:** This is the first genetic evidence to support that *M. ferruginea* black and *M. ferruginea* reddish brown are two distinct species.

500 plastome project: new tools for conserving the Pilbara flora

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Background: Effective identification of species is critical for conservation of biodiversity in the context of the challenges presented by changing land management practices, intensification in land use, and resource development. The Pilbara (north-west Western Australia) has a rich and diverse arid zone flora and is also an important region as a global source of iron ore. We developed a DNA database of Pilbara flora using high-throughput, low-coverage shotgun sequencing of whole chloroplast genomes and nuclear ribosomal sequences. The database will be used for accurate, timely, and cost-effective species identification, biodiversity monitoring, and restoration management. This will be a "living" dataset that will continue to grow as samples are added, new ways to analyse and use the data are developed, and new users contribute to the resource. **Results:** The project developed a DNA sequence resource comprising of 672 samples covering 577 named or proposed species. Substantial sequence information on the chloroplast genome (cpDNA) was obtained from 96.1% of the samples, and complete or near-complete sequences of the nuclear ribosomal RNA gene repeat (rDNA) were obtained from 93.3% of the samples. **Significance:** This project is the first large-scale Australian plant sequence database, developed with an open data framework. The database will link to global efforts to sequence organisms, and its open access nature ensures it will become a valuable tool for flora management in the Pilbara.

DNA barcoding of South African sponges

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Background: Southern Africa straddles two great oceans, which has created a large diversity of ecosystems that ranges from tropical coral reefs to cool-water temperate kelp forests. The region's shores are particularly rich in biodiversity, with >10 000 species of free-living marine animals having been recorded and described. This figure corresponds to 4% of the global fauna in practically all animal groups, but a very large number of species still remain to be described. This is particularly true of marine invertebrates, including sponges (Porifera). Taxonomic identification of sponges is based on morphological characters (color, size, shape, etc.) as well as the types of spicules they possess. These characters are considered to be insufficient to resolve sponge biodiversity, and in recent years, DNA barcoding has become a popular method to unambiguously identify different species. In the present study, we aimed to establish a reference library of DNA barcodes for sponges collected along the South African coastline. Further, we distinguished ecologically important, new and cryptic species by defining genetically distinct lineages using barcoding. Six hundred sponge samples were collected from different locations on the South, East, and West coasts of South Africa, and the COI gene was amplified. **Results:** We show that genetic differentiation among samples, considered to be the same species but collected at different locations, is considerable, indicating that levels of cryptic diversity are high and contributing to the growing evidence that South Africa's marine biodiversity has been vastly underestimated. **Significance:** Correctly identifying evolutionarily distinct lineages of sponges is important for understanding their biodiversity and discovery of pharmaceutically and biotechnologically valuable species.

A DNA barcoding approach to assess the risk posed by the aquarium trade in the spread of invasive aquatic plants in South Africa

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Background: The rise of invasive aquatic plants in South Africa's waterways is a significant environmental threat to the already fragile indigenous aquatic ecosystems and the services they provide. With the spotlight on aquarium and ornamental pond industries as the primary pathways by which non-native freshwater plants are introduced to South Africa, regulations such as the NEM:BA Act 10 of 2004 together with some national programs are currently in place to ensure compliance by the different role players. Despite these national initiatives, progress has been hampered by additional challenges, especially pertaining to species identification. As a result, we use a DNA barcoding approach as a tool to provide rapid and accurate identification of alien aquatic species currently in trade with the aim of providing a solution to border control officials to monitor plants coming into the country through various ports of entry. **Results:** We present results of the first DNA barcode reference library of traded aquatic flora. For 142 aquatic plants from nine aquariums around Johannesburg, ~90% could be identified to species level. Surprisingly, of all the traded species, 19% were categorized as 1a (Invasive Species), 8% as 1b (Invasive Species Controlled by Programme), and another 15% as prohibited under the NEM:BA Act. Additionally, more than 40% of these species originate from Asia, with a marginal 7% originating from other African countries. **Significance:** The results presented here have serious implications for the current nationwide invasive species management programmes. For implementation, a mixed technology solution has been developed in collaboration with the Biodiversity Institute of Ontario, Canada, and the Department of Environmental Affairs, South Africa (the LifeScanner Application), empowering the general community to identify suspect material.

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Illumina-based analysis of *Sorghum* fungal pathogens cultivated in vitro

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Background: *Sorghum* is an important crop in South Africa, but it is exposed to a variety of pathogenic fungi that cause many diseases, leading to significant losses in yields and reduction in quality of the product, thereby threatening food security. Although several control methods that have been applied have been successful, pathogens adapt and evolve quickly. Precise knowledge of the disease causal agents is essential for development of effective counter measures to fungal infections. The aim of the study was to survey local *Sorghum* fungal pathogens and use morphological and molecular approaches for identification. **Results:** *Sorghum* fungal pathogen diversity was investigated in 10 producing regions in South Africa using a culture-dependent technique. Morphological characteristics and ITS2 sequence data generated from Illimuna Miseq was used to identify the fungal isolates. A total of 258 isolates were obtained from leaf samples, from which operational taxonomic units belonging to 25 genera were identified as belonging to the phyla Ascomycota (92%) and Basidiomycota (8%). *Fusarium* was the dominant genus (30% of the isolates) followed by *Alternaria* (11%) and *Phoma* (9%). The diversity and abundance of different taxonomic groups differed with sampling locations. **Significance:** Studying the fungal pathogens, which cause diseases threatening the production of one of the important crops, *Sorghum* has a significant impact in crop production. A better understanding of these pathogens will lead to effective management and therefore food security for many living in poverty in South Africa. Pathogens cause diseases by producing enzymes that deconstruct the plant cell walls. Their ability to produce hydrolytic enzymes can be exploited for industrial bioconversion. The diversity of the fungi obtained in this study suggests that the enzymes produced are capable of providing the diversity and strength of activity required for bioconversion. This study will have a significant contribution in food security and in energy production.

Overview of Anjohingidrobe and Anjohimaletsy Bones Caves, Beanka Forest, Melaky region, western Madagascar

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Background: The Beanka Forest is characterized by karst geology that in some places are eroded, forming cavities such as Anjohingidrobe and Anjohimaletsy. **Results:** This study is a preliminary analysis of bone remains excavated in these caves. The obtained data were subjected to different analyses, such as biogeographic comparisons, Chi-square tests, and calculations associated with the index of specific diversity and dominance. The faunal similarity of the recovered bone material is similar between the two sites, and both contain numerous taxa belonging to the orders Amphibia, Reptilia, Chiroptera, Afrotheria, Rodentia, and Primata; Anjohingidrobe is the most diverse, and Anjohimaletsy contains extinct primates. **Significance:** The vastness of Anjohingidrobe Cave and associated moderated climatic conditions, provide better environments for bone preservation. The recovered bones show no signs of predation, whether animal or human. The cause of extinction of certain identified species is not yet clear and the subject of ongoing research.

DNA barcoding in the identification of biodiversity and studies in biogeography of marine–estuarine Teleostei fishes from Brazil

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Background: Knowledge of marine species diversity is relatively complete in families of commercial importance. However, gaps still exist in less-studied areas, such as some regions of the Brazilian coast. Several studies have shown that DNA barcoding is effective in identifying known and new species. This project aims to test the hypothesis of the existence of different genetic lineages of marine–estuarine fish along the Brazilian coast. To test this hypothesis, marine–estuarine fish species (demersal and pelagic) found along the Brazilian coast were examined by COI sequence analysis and morphological studies. **Results:** Nine species were analyzed: *Bairdiella rochus*, *Orthopristis ruber*, *Sphoeroides testudineus*, *Selene vomer*, *Chaetodipterus faber*, *Conodon nobilis*, *Hemicaranx amblyrhynchus*, *Lobotes surinamensis*, and *Nebris microps*. COI sequences were obtained for all species, and additional morphological studies were already done for several of these species. *Bairdiella rochus* was deeply revised, and three lineages were recognized: *B. ronchus* (revised); *B. veraecrucis* (resurrected); and a new species described from Brazil with two lineages, morphologically undistinguished until now. *Orthopristis rube*r and *S. testudineus* each presented two COI lineages and differences in morphology. The two lineages of *S. testudineus* are sympatric. *Lobotes surinamensis*, *C. nobilis*, and *N. microps* presented no differences in COI sequences in Atlantic South America, but the sequences differed from those of North America. *Conodon nobilis* showed morphological differences in South America. *Selene vomer*, *C. faber*, and *H. amblyrhynchus* presented no differences in COI sequences, and comparisons with Caribbean species are currently being conducted. However, *H. amblyrhynchus* presented differences in morphology along the Brazilian coast. **Significance:** These data show that there is still a significantly understudied diversity of marine fishes along the Brazilian coast that was selected by natural pressure processes driving the diversification in COI sequences, morphology, or both, in different magnitudes in different groups.

DNA banking and barcoding of neglected and underutilized leafy vegetables in South West Nigeria

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Background: Agricultural growth in Nigeria is increasingly recognised to be central to sustained improvement in economic development. However, in more recent years, there has been a marked deterioration in the performance of Nigeria's agriculture as the food production rate is insufficient to sustain its population. Indigenous leafy vegetables and fruits play a key role in income generation and subsistence, and they generate high economic returns per unit input be it land, water, or labour. Despite these values, these vegetables have been neglected for many years by researchers, policy makers, and funding agencies and are currently threatened with extinction. This project, therefore, aims at securing the genetic resources base of the neglected and underutilised leafy vegetables in South West Nigeria. Local communities in the south-west region of Nigeria were explored for sample collection representing eco-geographical distribution within the target area. Samples were identified using manuals and flora, and further authentication was done at the University of Lagos Herbarium. Total genomic DNA was isolated from all collected samples following the CTAB procedure with minor modifications. **Results:** A total of 23 leafy vegetables were collected; of these, 18 species are indigenous to the study area, while 5 species are indigenous to the

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southern region of Nigeria. The most abundant were members of the family Amaranthaceae. Voucher specimens of collected samples were deposited at the University of Lagos Herbarium, in Lagos. The extraction process yielded quality DNA samples that have been deposited in the DNA Bank at the University of Lagos. DNA barcodes were generated using *rbcL* and *matK* genes and deposited at GenBank. The barcode sequences are being analysed. **Significance:** This study can be seen as a basis upon which further research on the neglected and underutilised vegetables can be based.

Is molecular evolution faster in the tropics?

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Background: The evolutionary speed hypothesis (ESH) suggests that molecular rates are higher among species inhabiting warmer environments compared with those in cooler temperatures. Previously, the ESH has been investigated using small numbers of latitudinally separated sister lineages. In animals, these studies typically focused on subsets of Chordata and yielded mixed support of the ESH. Thus, a more diverse selection of lineages from across the animal kingdom is needed to further test this hypothesis. **Results:** Using a novel bioinformatics pipeline written in R (publicly available at https://github.com/m-orton/R-Scripts), this study analyzed public COI barcode data from the Barcode of Life Data System (BOLD) for six of the largest animal phyla (Arthropoda, Chordata, Mollusca, Annelida, Echinodermata, and Cnidaria) and paired latitudinally separated taxa together in an automated fashion. Barcode Index Numbers (BINs), a molecular proxy for species, were paired that were separated by at least 20 degrees in median absolute latitude with between 0.02 and 0.15 pairwise sequence divergence. Of 8352 lineage pairs, 4327 (51.8%) displayed a higher molecular rate in the lower-latitude lineage compared with 4025 (48.1%) with a higher rate in the higher-latitude lineage. Overall, a weak trend was found supporting higher rates of COI evolution in lineages inhabiting the tropics versus those inhabiting cooler regions. Arthropoda and Chordata exhibited higher rates of molecular evolution at lower latitudes significantly more often than expected by chance, with binomial test *p*-values of 0.007 and 0.009, respectively. The strongest trend was observed in Echinodermata, although the sample size was modest for that phylum. **Significance:** To date, this study represents the most comprehensive analysis of latitude-related molecular rate differences across animals. While a statistically significant pattern was detected, the overall weak support for the EHS, when considering all taxa, suggests that the EHS may not serve as a universal mechanism underlying the latitudinal diversity gradient. This study also highlights the merits of using automation to analyze large DNA barcode datasets.

Resource partitioning among large herbivores and structure of plant–herbivore interaction networks in savanna: new insights from fecal DNA

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Background: Understanding ecological processes controlling large mammal herbivores (LMH) coexistence and plant–herbivore interactions is a crucial issue for the conservation of savanna ecosystems. Architectural characteristics of ecological networks (e.g., connectance, modularity, etc.) are fundamental components of the stability of communities. Hence, the structure of the plant–herbivore interaction networks, and the degree of resource partitioning among LMH species, are important parameters for the stability of savanna ecosystems. However, disentangling factors influencing the dietary niche overlap among herbivores and trophic network characteristics remains challenging due to the lack of precise large-scale dietary data. Recent advances in DNA (meta)barcoding and next-generation sequencing (NGS) have led to the development of new approaches to precisely characterize animal diets from DNA retrieved in dung samples. In this study, we implemented a fecal DNA metabarcoding approach in multiple African protected areas to determine large herbivore diet and illuminate ecological processes structuring plant–herbivore interactions in savanna. **Results:** Dung samples from common large herbivores have been collected in different protected areas through Africa. DNA-based dietary data enabled to (*i*) determine precisely the diets of LMH, (*ii*) assess the degree of niche overlap among this guild, (*iii*) describe the structure of plant–herbivore interactions, and (*iv*) investigate factors influencing characteristics of trophic networks pivotal for the stability of the system. These results highlight how abiotic parameters and some biotic components of the ecosystem (e.g., predation pressure) influence plant–herbivore interaction networks and ungulate community assemblage rules. **Significance:** The structure of ecological networks can be altered by anthropogenic pressure, and these alterations may have an important effect on ecosystem functioning. In addition to providing useful information for the management of game species, these data allowed to determine the fine-scale structure of plant–herbivore interaction networks and their local drivers. These results provide new insights on mechanisms involved in community assemblage and stability of savanna ecosystems.

Forensic botany and forensic chemistry working together: advances on applications of plant DNA barcoding in complementing some specific demands of forensic sciences in Brazil. A case study

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Background: Forensic chemistry is a popular and widely accepted technique for the assessment of suspected plant material in forensics. Joining reliability, speed of analysis, cost, and relatively effortless bench work, it is a gold standard for evaluating plant material suspected to be an illicit drug. Notwithstanding, sometimes it can be a limited technique by the low levels or absence of chemicals upon which analysis is based, e.g., in cases involving seeds, tiny amounts of material and many other situations. This is the present case: where illicit drugs or controlled chemicals were not found. As the aforementioned suspicion remained, we had to appeal, therefore, to the plant DNA barcoding technique. **Results:** Using multiple sets of primers (7 pairs), barcodes were obtained from 10 specimens, representing 5 taxa from 2 packets of botanical material, at first sight, homogenous. Most of the specimens (4 samples) were of the same nature, which we could identify to species level (*Artemisia absinthium*). **Significance:** At this degree of complexity, it is the first report about an effective effort using an association between forensic botany and forensic chemistry techniques in order to assess the real nature of a suspected plant material in Brazil. Such an approach was done mainly due to claims from the national judiciary system. Notwithstanding the rigorous and routinely implemented practices in forensic sciences, sometimes it is imperative to lay hand on techniques not routinely employed in order to satisfy the final client on its questions. This case, thus, can serve as salutary self-criticism about the frequent tendency of least effort in analysis. The combination of DNA barcoding with forensic chemistry, therefore, will always offer an efficient solution to evaluate plant material adequately from now on.

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DNA barcoding halictine bee species from Europe and Africa

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Background: The Halictinae constitute a large subfamily of bees with more than 150 species in Europe and about a hundred species in Africa. Recently, DNA sequences were published for halictine bees mainly from the New World, but also from Central Europe (62 species) and Africa (89 species). However, for many groups of African and Mediterranean species, identification and classification remain exclusively based on morphological traits. Here, we aim at collecting DNA barcode data for those halictine species with a large collection of specimens available at the Royal Belgian Institute of Natural Sciences and the Royal Museum for Central Africa. Our goals are to improve the resolution of the species identification of African and European halictine species, to compare their DNA barcodes with those already collected, and, in combination with sequencing four nuclear genes for some key species, to classify the species to their respective subgenera. **Results:** Genetic sequences are currently analyzed for a set of 172 specimens representing 150 species. Most of the groups represented by these species have not been included in previously published phylogenetic or barcoding studies. **Significance:** Our research is a renewed effort to compile DNA barcodes from a large number of specimens representing all the subgenera of halictine bees across a broad geographic area of the Old World (Europe and Africa). It should allow us to propose an updated classification of the species of the Old World Halictinae in concordance with the subgenera proposed for the New World.

Ecobarcoding: taxonomy-free approach for high-throughput environmental DNA-based biomonitoring

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Background: Monitoring biodiversity is essential to assess the environmental impacts of increased anthropogenic activity. Traditional biomonitoring involves the sorting and morphological identification of organisms, which is cost and time consuming. Several recent studies have shown that environmental DNA (eDNA) metabarcoding can be used as an alternative to morphotaxonomy-based biomonitoring. In all these studies, eDNA sequences were assigned to morphospecies of known ecology. However, the incompleteness of the DNA-barcode reference database and frequent conflicts between molecular and morphological data often impede the correct assignment of eDNA sequences. **Results:** To overcome these limitations, we investigated the possibility to use a taxonomy-free approach that would provide ecological values for eDNA sequences (ecobarcodes) without any reference to morphotaxonomy. We applied this approach in the case of two groups of bioindicators: diatoms and Foraminifera. We used different computing approaches, including supervised machine learning, to predict biotic indices from eDNA data. Our study yielded very promising results by providing similar ecological status as obtained from morphotaxonomy-based surveys. **Significance:** The main advantage of this approach is that almost the entire eDNA dataset can be used instead of only those sequences that could be assigned to morphospecies. Its main limitation is that the method has to be calibrated based either on independent bioassessment or chemical parameters. However, once calibrated, the taxonomy-free approach can be easily standardized and applied in routine biomonitoring, as a complementary tool allowing fast and cost-effective assessment of environmental impacts.

Molecular barcodes for Philippine *Bactrocera dorsalis* and *B. occipitalis*: insights for pest management through identification

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Background: The *Bactrocera dorsalis* complex (Diptera: Tephritidae) are widely known fly pest species of fruiting trees. While this group exhibits plastic morphologies and has a widespread distribution, the current taxonomy of the complex has been resolved through phylogenetics and cladistics. Application of this resolution, however, still needs to be translated to communities most affected by these pests. Molecular identification is a potential method for rapid identification as conventional taxonomy of the complex is difficult. This study aims to evaluate the barcoding genes COI, 16S, and 18S of *B. dorsalis* in the Philippines to determine a suitable marker for identification using the NCBI GenBank database and molecular phylogenetics. **Results:** *Bactrocera dorsalis* and *B. occipitalis* were caught in methyl eugenol traps in localities spanning Luzon, Cebu Island, and Zamboanga. Representative samples (n=7) from each locality were subjected to DNA barcoding. The 18S rRNA gene did not show any sequence difference between the species, while the 16S rRNA fragment was able to distinguish *B. dorsalis* from *B. occipitalis* through a single SNP. The COI gene was highly polymorphic; however, geographical clustering did not occur within species. BLAST results for the COI gene fragment were mostly inconclusive due to multiple species hits for the haplotypes. Phylogenetic analysis of the concatenated COI and 16S genes gave a more accurate species identification that was in concurrence with morphological analyses of the samples. **Significance:** The lack of local databases for pest species in the Philippines is a hindrance for the development of integrated pest management. Current updates in taxonomy of the *B. dorsalis* complex needs to be translated to the local farmers through a more efficient way of identification. Barcoding and phylogenetic analyses of the COI and 16S gene fragments have been found to effectively delineate species and should be explored for other tephritid pest taxa.

From mitochondrial genes to genome: updating barcodes in domestic animals

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Background: The traditional DNA barcoding technology, based on several mitochondrial DNA (mtDNA) genes, is used to identify species. They usually cannot provide enough information to dissect the lineages within populations, within (sub-)species levels. This pitfall is magnified in mtDNA studies within domestic animals. It is mainly due to most domestication events having occurred within the Holocene. The accumulation of genetic differentiation is small, in such a short evolutionary history. Within the past decade, mitochondrial genome sequencing has been applied in various domestic animals. And the strategy of haplogroup tree analysis has been adopted to reconstruct the genealogy or hierarchy of mtDNA lineages. **Results:** We develop DomeTree (http://www.dometree.org), the most up-to-date mtDNA haplogroup tree and a standardized hierarchical haplogroup nomenclature system for cattle, dogs, goats, horses, pigs, sheep, yaks, and chickens. In addition, we provide the software MitoToolPy (http://www.mitotool.org/mp.html) to facilitate the analyses of

mtDNA fragments and genomes. **Significance:** The updated mtDNA phylogeny including its haplogroup nomenclature can serve as a starting point for future mtDNA analyses in domestic animals. Our toolkit also serves as a convenient barcoding tool for strains or breeds of domestic animals. It is used not only in analyses of genetic diversity and domestication history but also in investigations of food authenticity and non-human forensics.

DNA barcodes for Canadian beetles: high identification success and insights into the Holarctic fauna

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Background: Beetles (Coleoptera) are one of the most diverse animal taxa. Nearly 400 000 species have been described, and at least as many are thought to await discovery and description. Among the 8200 species of beetles currently known from Canada and Alaska, many are thought to be shared with Eurasia. Recent progress in barcoding the European and the Canadian beetle faunas provides an opportunity to both explore diversity within supposedly Holarctic species and discover new Holarctic or adventive species. **Results:** By March 2017, DNA barcodes were available for 4596 named Canadian beetle species. In addition, several hundred Barcode Index Numbers (BINs) lack a species- or even genus-level identification. More than 90% of named species are unambiguously identifiable based on DNA barcodes. Cases of BIN sharing between species are rare, and barcode haplotypes shared between species are even rarer. The ongoing cleaning and validation of the data has already revealed that many, probably most, of these cases reflect misidentifications. Several BINs with Holarctic distributions either have no name in Canada or have different names in Canada and Europe despite in some cases sharing haplotypes, reflecting new Holarctic or adventive species and possible synonymies or misidentifications. Conversely, in several cases, taxa assigned to the same species in Europe and Canada show deep sequence divergence. The latter results indicate that many species currently thought to be Holarctic are likely pairs or complexes of species. **Significance:** DNA barcodes have been demonstrated to be highly useful in species identification and discovery in European beetles. The current study indicates that the same situation applies to the Canadian fauna. Comparing and combining two major regional beetle barcode libraries will greatly advance our knowledge of both faunas, and helps to flag cases where re-evaluation of the current taxonomy is needed.

Mapping terrestrial biodiversity across the planet: a progress report on the Global Malaise Program

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Since its initiation in 2012, the Global Malaise Program (GMP) has continued to gather extensive biodiversity data across the planet through a standardized protocol of Malaise trapping and DNA barcoding. This ongoing international collaboration has effectively collected arthropod specimens from 63 sites in over 40 countries. Malaise samples from 44 locations have been processed with an average of 28 weekly samples sequenced per site. In total, 1.16 million specimens have been analyzed to date, resulting in ~992 000 barcodes and 110 000 Barcode Index Numbers (BINs, a proxy for species). Over half of these BINs (70 000) are only found in the GMP dataset compared to the rest of the Barcode of Life Data System (BOLD), and nearly 51 000 BINs are singletons. This latter figure, and the near-linear specimen-based accumulation of BINs at most sites, indicates that substantial diversity remains undetected. The expansion of the BOLD reference library, through the barcoding of identified material and morphological examinations by taxon experts, has increased our capacity to assign taxonomic identifications to unidentified specimen records regardless of location. Using the BOLD Identification Engine, we have now assigned at least a family-level identification to 90% of all GMP BINs through taxonomy matches by BIN and through barcode neighbour-joining trees. Along with comparing overall diversity between GMP sampling sites, we can now delve deeper into the data to investigate relationships between diversity indices and environmental variables such as temperature, precipitation, and habitat. It is also now possible to probe certain taxonomic groups with significant environmental relevance, leading to a more accurate evaluation of anthropogenic impacts. Going forward, GMP will continue to expand this global and globally-unique dataset, and investigate the patterns it reveals.

Bacterial diversity in long-term As-contaminated technosols (Zemianske Kostolany, Slovakia)

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This study is focused on the isolation and identification of autochthonous bacteria in arsenic-contaminated soils from Zemianske Kostolany (Slovakia). Studied technosols represent a unique system of a 50-year-old environmental burden after a dam failure of a coal-ash pond. The released ashes rich in arsenic with a thickness of 1–2 m were covered by a 40 cm thick layer of soil. Long-term exposure and selection pressure of elevated concentrations of arsenic (a range of 93–634 ppm) induced the formation of the specific adapted autochthonous microorganisms. Based on phylogenetic analysis, isolated bacterial strains were composed of four phyla and represented by common strains in soils: Proteobacteria (60.9%), Firmicutes (21.7%), Actinobacteria (8.7%), and Bacteroidetes (8.7%). The phylum Proteobacteria was represented by the species *Pseudomonas baetica*, *P. fluorescens*, *P. chlororaphis*, *P. koreensis*, *P. putida*, *P. reinekei*, and *Pseudomonas* sp. The phylum Firmicutes was represented by the species *Bacillus cereus* and *B. pumilus*. There were further recorded two isolates of the genus *Chryseobacterium* (Bacteroidetes). The genera *Streptomyces* and *Rhodococcus* (Actinobacteria) were represented by only one species. Results of studied bacterial species diversity, which was able to adapt to living in a contaminated environment, are the basis for the application of selected indigenous bacterial species in the bioleaching process as one of the potential methods for bioremediation of arsenic-contaminated soils.

Intraspecific sample size estimation for DNA barcoding: are current sampling levels enough?

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Background: The determination of adequate sample sizes for successful species identification has long been recognized as vital since the early days of DNA barcoding. However, deep taxon sampling is often secondary to maximizing the number of different taxa sampled. While general consensus points to the sampling of 5–10 individuals per species as sufficient for most phylogeographic barcoding applications, this figure is highly constrained by the occurrence of rare species and unclear species boundaries. This talk will present a novel statistical model to predict adequate sample sizes necessary to uncover the majority of species DNA barcode haplotype diversity, given observed numbers of unique haplotypes and specimen sequences. 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 gauged through the use of haplotype accumulation curves, in order to determine the value on the x-axis where haplotype saturation occurs. The proposed model will be framed in

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the context of investigating COI haplotype variation in the ray-finned fishes (Chordata: Actinopterygii). **Results:** Generated haplotype accumulation curves showed evidence of asymptotic behaviour for only three of the 18 examined fish species. The model finds that 150–5400 specimens per species are likely needed to recover all estimated haplotype diversity. **Significance:** The present null model is expected to work best for already well-sampled clades. As such, it can be considered to be a worst-case scenario for specimen sampling. While model estimates may not be practical, it offers a glimpse into the most appropriate taxon sample sizes to target. This work has tremendous implications for accelerating the growth of DNA barcode reference libraries for molecular species diagnosis, as well as aiding the calculation of barcode gap thresholds for species delimitation purposes.

High-throughput classification of COI metabarcodes using a naive Bayesian classifier

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Background: Groups that have used traditional biomonitoring methods for determining ecosystem status have started to incorporate COI metabarcoding in their workflows to facilitate monitoring in a more cost-effective and time-efficient manner. Until now, there has been difficulty with assigning names to COI partial barcodes in a rapid, high-throughput manner, while simultaneously providing a statistical measure of confidence for each assignment. **Results:** We have compiled a reference library of 912 253 COI sequences mined from the GenBank nucleotide database. This reference set can be used to classify chordates, arthropods, and flag other members of complex eDNA communities as belonging to other major eukaryote groups. We adopted the well-known taxonomic assignment tool, the naive Bayesian classifier available from the Ribosomal Database Project, to enable high-throughput COI taxonomic assignments. We provide statistical support cutoff guidelines for COI fragments of different sizes. We also test the coverage and classification accuracy, in silico, of a variety of COI fragments generated from primers in the literature. We directly compare run-time and false positive rates generated from using the naive Bayesian classifier or the commonly used top BLAST hit method. **Significance:** We show how the naive Bayesian classifier can be used to analyze freshwater and benthos communities detected by COI metabarcoding. We demonstrate the advantage of using a purpose-built taxonomic assignment tool over using the more general, but still widely used, top BLAST hit method to facilitate high-throughput taxonomic assignments in a reasonable time frame and to reduce rates of false positive assignments.

DNA barcodes as tools for studying phylogenetic structure in the New Zealand grass flora (Poaceae)

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Background: Insular floras that contain high levels of endemism and exotic species naturalisations provide fascinating case studies of DNA barcoding and phylogenetic structuring. Rich assemblages of historically accumulated indigenous diversity (often including radiations of taxa) and recently arrived naturalised diversity (often including taxa closely and distantly related to natives) offer unique challenges and insights. The grasses of New Zealand—a species-rich flora consisting of 161 endemics, 33 indigenous non-endemics, and ~230 naturalised exotics (~420 in total)—represent such a group. Native diversity is distributed across 12 of the 51 currently accepted tribes of Poaceae, but taxa from two tribes comprise 84% of the species: Poeae (116 spp.) and Danthonieae (47 spp.). Naturalised diversity, by contrast, is more evenly distributed across 20 tribes. **Results:** A three-locus DNA barcoding library (*rbcL*, *trnL-F*, nrITS) was assembled for 402 species across 124 genera, combining both newly generated and publicly available sequence data. Results show these loci are capable of identifying most species in the flora (>75%), but identifications in recently radiated taxa were often indeterminate (e.g., *Chionochloa*, *Rytidosperma*, *Festuca*). Phylogenetic trees produced from the data were well-resolved across the broader grass phylogeny. Phylostructure comparisons between the native and exotic components show the structuring of the naturalised flora congruent with a neutral hypothesis, while the indigenous flora was found to be more clustered than expected by chance. **Significance:** This is the first DNA barcode library constructed for the grasses of New Zealand. The results improve our understanding of Poaceae barcoding in a floristic context and provide an example of a phylogenetically clustered native grass flora. Future research is planned to use this framework to test Darwin's naturalisation hypothesis (i.e., introduced taxa are more successful in areas where their close relatives are absent).

To network, or not to network, that is the phylogenetic question

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The bifurcating model of evolution, which is the foundation of phylogenetic trees, performs poorly when faced with biological violations of the assumption of exclusive divergence; the likelihood of violations such as hybridisation events increases as one nears the tips of the tree of life. Solutions to capturing and understanding non-bifurcating evolution often lie in an array of available phylogenetic networks, yet these are rarely used. This is often due to the conceptual (and methodological) complexities surrounding networks. In this talk, I build on the methods aimed at transferring information between trees and networks, proposed by Schliep et al. (2017, Methods Ecol Evol. doi: 10.1111/2041-210X.12760). This includes a framework to understand the complex array of network types, how to make direct comparisons among trees and network types, and provide case studies to highlight the utility of this framework and data transference for exploratory data analysis and hypothesis testing.

Decoding ice-plants: challenges associated with barcoding and phylogenetics in the diverse family Aizoaceae

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Background: Aizoaceae are a large, diverse family in the Greater Cape Floristic Region of South Africa. Five subfamilies are recognised, viz. Aizooideae, Mesembryanthemoideae, Ruschioideae, Sesuvioideae, and Tetragonioideae, with Ruschioideae being the largest and most recently diverged. Phylogenetic relationships in Aizoaceae are notoriously poorly resolved, and up to 10 plastome gene regions are required in Ruschioideae to build informative phylogenies. Multiple copies render nuclear regions problematic, while low-copy nuclear regions tested are not successfully sequenced without cloning. Identification of taxa using morphology is challenging as taxa are mainly distinguished by specialised fruit characters and make poor herbarium specimens. Barcoding as an aid to identification may be advantageous but has never been investigated. **Results:** The challenge to produce resolved phylogenies is evident when comparing published phylogenies of Ruschioideae and Mesembryanthemoideae. In the former, 21.4% of characters in the combined matrix of three plastid regions were parsimony informative, while in Ruschioideae only 7.5% of characters in the combined matrix of 10 plastid regions were parsimony informative. The percentage of parsimony-informative characters of plastid regions were further explored in an expanded phylogeny of the *Conophytum*-clade (Ruschioideae). The relative number of variable

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characters in each of the six plastid gene regions (*matK*, *rpl*16, *rps*16, *trnL-F*, *trnQ-rps*16, *trnS-trnG*) ranged from 4.0% to 13.64% and percentage of parsimony informative characters from 2.8% to 8.1%. The barcoding region *matK* was shown to be the least variable, with the fewest parsimony-informative characters. Results of further sequence variation parameters explored will be presented. **Significance:** This study provides insight into sequence variation and the contribution to the resolution of the plastome gene regions currently used in Aizoaceae phylogenetics. This forms part of a larger study that will explore whole-genome sequencing to identify hypervariable regions in the plastome and nucleus, which may be used to produce phylogenies with improved resolution and identify additional barcoding regions.

Determining the level of substitution in herbal products containing *Harpagophytum* spp. through a standard reference barcode library

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Background: *Harpagophytum procumbens* (the preferred species) has traditionally been used as a treatment for inflammation, fever, and, in some cases, malaria. Due to the commercial demand and unsustainable harvesting techniques, the industry is subjected to the possibility of substitution with the more inferior species, *H. zeyheri*. Granting that several pharmacopeias allow for the use of either *H. zeyheri* or *H. procumbens*, the pharmacological effect on consumers (patients) and the equivalence of this interchangeable use has not been studied. The industry is starting to explore DNA barcoding as a method for quality control of botanical medicines. **Results:** In this study, we explored the potential application of DNA barcoding to determine authenticity in commercial products. Authentic botanical reference material of both *H. procumbens* (*n*=30) and *H. zeyheri* (*n*=20) were obtained. A total of 10 commercial products were purchased on the internet in 2016 using the search term "Harpagophytum" or "Devil's Claw". The two barcoding regions (*rbcLa* and *matK*) and the additional plastid region *trnL-F* was first used to construct a standard reference barcode library for the genus *Harpagophytum,* and second to barcode the purchased herbal products claiming to contain *Harpagophytum.* The barcode library was able to authenticate all commercial products (query samples) up to species level. Furthermore, the character-based (BRONX) analysis was performed to verify taxonomic identity of the query samples. BRONX results indicated that 69% of the commercial samples tested, labeled as *H. procumbens*, were substituted with *H. zeyheri*. **Significance:** Our study is the first to construct a reference barcode library for *Harpagophytum*. This approach of DNA barcoding could significantly support the authentication of herbal products containing species of *Harpagophytum*.

DNA barcoding and wildlife enforcement: identification of animal and plant derivatives through high-throughput sequencing

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Background: Illegally trafficked wildlife products are often intercepted at national borders, but objects suspected to be made from CITES-listed species can be difficult or impossible to identify visually. Consequently, DNA-based identification is being employed to ascertain the species composition of confiscated objects, which is hindered by samples potentially containing DNA from multiple sources. Additionally, the DNA itself may be heavily degraded due to age, chemical, or thermal processing, or attempts by traffickers to mask the true origin of the object. A definitive answer is required within legally prescribed time frames, so analysis must be thorough and not exceed 2–3 weeks. **Results:** We use DNA barcoding to identify items confiscated by Canadian authorities. The items included powdered supplements, creams, oils, spirits, dried organs, bone, ivory, teeth, keratin, egg shells, sea shells, corals, scales, feathers, dried skin, taxidermied specimens, tanned hides, and textiles. To assess the feasibility of rapid, high-throughput genetic analysis, we subjected the items to different types of DNA extraction methods, primer sets, and sequencing platforms ranging from Sanger sequencing to second- and third-generation sequencing. Our results show that the choice of DNA extraction method is important when analyzing different types of material. Furthermore, primer sets must be carefully chosen to account for diversity and DNA degradation, yet produce an amplicon of sufficient length for adequate taxonomic resolution. Finally, while Sanger sequencing permitted single-source item identification in many cases, the sensitivity of next-generation sequencing coupled with its ability to separate mixed sources was required for half of the objects in this study. **Significance:** We address the critical need for a reliable and reproducible DNA-based species identification method that complies with legal requirements, chain-of-custody procedures, and timeline standards established for wildlife enforcement at ports of entry.

Using plant DNA barcoding markers for the identification and detection of peanut, a major allergen in food products

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Background: Peanut (*Arachis hypogea*, Fabaceae) is one of the most consumed legumes in the world, and it plays an important role in human nutrition, given its high protein and fat content. Peanut allergy, however, is one of the most severe food allergies because of its persistence throughout the lifetime of individuals and its severity, even at very low doses. With the aim of preventing accidental consumption by allergic individuals, the U.S. Food and Drug Administration has been developing methods to effectively identify the presence of peanut in food. In this study, we evaluated the utility of the DNA barcoding markers to develop real time PCR assays that detect this allergen in a broad range of food samples. **Results:** As part of a thorough survey, we have tested nine different loci that have been previously used for plant and allergen identification: the nuclear genes *Ara h 1* and *Ara h 2* (allergen protein genes), the Internal Transcribed Spacer (ITS) 1 and ITS 2, and the chloroplast regions *rbcL*, *matK*, *rpl*16, *trnL*, *trnL-F*, and *trnH-psbA*. Our results show that the chloroplast markers provide the most specific and sensitive detection, even at trace levels. **Significance:** Food allergies have become an increasing concern to public health worldwide. To help protect consumers in the United States, the Food Allergen Labeling and Consumer Protection Act of 2004 requires the presence of any of the major food allergens (including peanut) be declared on the label. Nonetheless, allergic reactions can be triggered by accidental consumption of mislabeled or cross-contaminated food products. DNA-based methods have become an important tool to accurately identify and detect these allergens and support the enforcement of this regulation.

High-throughput plant DNA barcoding using microfluidic PCR: a new method for referencing the tree of life

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Background: Plant DNA barcoding routinely uses more than one locus for species identification (*rbcL*, *matK*, *psbA-trnH*, and nrDNA ITS), which significantly increases sample handling, preparation time, and

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costs. Different primer pair combinations (~12) are necessary to amplify DNA barcode sequences from all major lineages of vascular plants, from seedless to flowering plants. Most of the publicly available data are from flowering plants, while groups such as gymnosperms, ferns, and lycophytes are underrepresented or absent, thus creating a significant gap in the reference libraries for plants. Microfluidic PCR using the Fluidigm Access Array can optimize plant DNA barcoding by simultaneously amplifying targeted regions for as many as 48 DNA samples and up to 480 PCR primer pairs (a total of 23 040 PCR products) during a single thermal cycling protocol. High-throughput DNA barcoding would also support a more accurate taxonomic identification of poorly known plant groups and improve our understanding of plant diversity. **Results:** As a proof of concept, we developed a microfluidic PCR workflow using the Illumina Miseq platform to generate new sequences for each of the four DNA barcode loci in plants (384 total sequences), and to build a reference library that includes 77 families and 96 genera from all major plant lineages including bryophytes, ferns and lycophytes, gymnosperms, and all major groups of angiosperms that are currently lacking in the public databases. Our results showed that this technique was twice as fast, almost half the cost, and generated a barcode library with a more comprehensive taxonomic coverage. **Significance:** Microfluidic PCR offers a highly efficient alternative compared to traditional PCR and Sanger sequencing. It also provides the opportunity to apply high-throughput sequencing methods to optimize the generation of DNA barcode data and make plant DNA barcoding more accessible to a wider community for far-reaching applications.

DNA barcoding of the walking catfish, *Clarias batrachus* (Linnaeus, 1758), reveals presence of cryptic species and corrects misconception about its status in the Philippines

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Background: The freshwater catfish, *Clarias batrachus*, is a food fish that is widely distributed in Southeast Asia. It has also been reported from South Asia and China. It is widely believed to be an introduced species in the Philippines going back to a reported introduction from Thailand in 1972. **Results:** Five specimens were DNA barcoded from each of four Philippine lakes and two river drainages using cytochrome *c* oxidase subunit I (COI) and cytochrome *b* (*cyt b*) genes. Additional COI and *cyt b* sequences were mined from GenBank. A neighbour-joining tree using the Kimura 2-parameter (K2P) method showed that Philippine COI sequences including 28 of the 30 sequences from this study formed a single clade (Cluster 1). The two remaining sequences from this study (CBT37 and CBT46) clustered with Malaysian sequences (Cluster 3). Sequences from Thailand formed a separate clade (Cluster 2). Indonesian samples formed another cluster (Cluster 4). K2P distances ranged from 0% to 0.2% for Cluster 1, 0% to 0.4% for Cluster 2, 0% to 1.6% for Cluster 3, and 0% to 0.4% for Cluster 4. The distance between the sub-cluster containing samples CBT37 and CBT46 and sequences from Cluster 1 (all other samples from the Philippines) had a range of 4%–4.7%. The average distance between the Philippine cluster and the Thailand cluster was 2.3%. **Significance:** This study revealed the presence of cryptic species within *C. batrachus* in the Philippines. The genetic uniqueness of the majority of Philippine *C. batrachus* specimens is supported by historical records of its presence in the Philippines. A review of the literature showed that even prior to the reported introduction from Thailand in 1972, *C. batrachus* was a native and widespread species in the Philippines. Albert Herre even listed it in 1927 as one of the true freshwater fishes of the Philippines.

Comparative authentication of *Hypericum perforatum* L. (St. John's wort) herbal products using DNA metabarcoding, TLC, and HPLC-MS

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Background: *Hypericum perforatum* L. (St. John's wort or Guan ye Lian Qiao) is a top-selling over-the-counter (OTC) product. Despite its long history of use to treat mild to moderate depression, there is an increasing concern over the product's efficacy, safety, and quality in the wake of recent cases exposing discrepancies between labeling and actual constituents. The use of substitutes and unlabeled fillers is driven by the lack of standardized methods for quality assessment and the highly competitive market, but also by accidental adulteration, misidentification, or mixed-up plant species nomenclature. The traditional quality assurance of herbal products is based on macroscopic and microscopic characterization, phytochemical analysis of therapeutic target compounds, and assays for toxic constituents such as heavy metals and toxins. However, these products are usually highly processed and multi-ingredients, and these methods might not accurately identify all plant ingredients. To complement these methods, the European Medicines Agency (EMA) and United States Food and Drug Administration (FDA), support the use of innovative analytical technologies, such as DNA barcoding. **Results:** In this study, we used amplicon metabarcoding (AMB) to authenticate 78 *H. perforatum* herbal products and evaluate its ability to detect substitution compared with the current identification approaches, such as thin-layer chromatography (TLC) and high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Using AMB, *H. perforatum* was detected in 68% of the products, and the substitution, adulteration, and (or) admixture of other species was simultaneously identified. TLC and HPLC-MS are accurate methods for authenticating the presence of target chemical compounds, but they showed limited efficiency in unambiguously detecting *H. perforatum*; also, they do not yield any information on other plant ingredients in the products. **Significance:** Post-marketing AMB of herbal products by regulatory agencies would provide an incentive to manufacturers to increase quality control from raw material to commercialized products.

Shared informatics infrastructure advancing barcoding efforts in marine environments

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Background: DNA barcoding has been shown to be an effective tool for species identification and discovery in marine eukaryotic organisms. Efforts to date have resulted in the addition of 27 000 marine species to the DNA barcode library. However, existing catalogs count nearly 228 000 eukaryotic species, and recent estimates suggest up to 2 million species live in the oceans— a considerable distance to go before completing the library. Working in marine environments pose financial and logistical challenges beyond those for terrestrial or freshwater environments, making it difficult to scale efforts. Such challenges make it necessary to be more considerate in establishing sampling strategies that would enable us to capture and catalog the widest diversity. Tools like the Barcode of Life Data System (BOLD) have accelerated barcoding efforts by providing shared infrastructure for data storage and analyses of barcode data. We seek to take this further by integrating data from BOLD with other biodiversity data sources to build a federated database to guide further barcoding ef-

forts. **Results:** We performed a gap analysis on the barcode library by integrating data from World Register of Marine Species (WoRMS) with BOLD. Through this federated database we obtained the marine species yet to be barcoded and resolved current synonymies. In addition, we identified biodiversity hotspots in the oceans where the greatest diversity of species have been collected. WoRMS shows 228 000 species of marine eukaryotes, with 27 000 matching those in BOLD. **Significance:** BOLD and other informatics platforms can enable tracking and analysis of marine biodiversity patterns. Taking advantage of these resources can help produce accurate data on marine biodiversity, leading to better predictions about global changes in the oceans. We provide an exemplar case by using these resources to develop a hit list of marine species to be barcoded and locations where they may be found.

Genetic characterization of freshwater fishes in Bangladesh using DNA barcodes

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Background: Bangladesh is a biogeographically important area in the heart of the hyperdiverse Indo-Burman region of South Asia, but it has one of the most taxonomically unresolved freshwater fish faunas in the world. Although substantial progress has been made in documenting fish species diversity in Bangladesh based on morphological studies, the diversity of fish species has not been fully explored. This project focuses on genetic characterization of Bangladesh's freshwater fish fauna in the form of a DNA barcode library composed of standardized, well-identified mitochondrial COI sequences based on taxonomic revisions and deposition of voucher specimens, to ensure stability in nomenclature used in the database. **Results:** A reference database has been developed based on the mitochondrial cytochrome *c* oxidase subunit I (COI) sequences of >200 species of Bangladeshi freshwater fishes. Barcode sequences are submitted to GenBank following taxonomic validation. To date, two new species, namely *Danio annulosus* (3.4% p-distance from the most similar species) and *Garra mini* (12% p-distance from closely related taxa available in GenBank) were described in combination with morphometric studies. A rapid expansion of several alien species (e.g., *Trichopsis vittata, Pterygoplichthys disjunctivus*) has also been detected. The barcode sequences from the present study along with traditional taxonomy have also confirmed the existence of many misidentifications in current literature. **Significance:** This is the first comprehensive attempt to develop a DNA-based reference library for freshwater fishes of Bangladesh that provided several new species, new records, and high taxonomic resolution of existing taxa, improving on previous taxonomic identifications. This research will result in improved species detection and tools to determine priority areas for conservation or management of freshwater communities. This study also underscores the scope of further investigation into surveillance of fish species composition and invasive alien species using environmental DNA.

Barcoding of Moroccan *Dactylogyrus* (Monogenea: Dactylogyridae)

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Background: Cyprinidae is one of the most speciose families of freshwater fishes. In Morocco, continental waters are dominated by cyprinids. Currently, 18 species are known, belonging to four genera: *Luciobarbus* Heckel, 1843, *Carasobarbus* Karaman, 1971, *Labeobarbus* Rüppel, 1835, and *Pterocapoeta* Günther, 1902. Species of *Dactylogyrus* are gill parasites that are almost exclusively found in cyprinids. These parasites can be used as specific markers as well as population markers. **Results:** To extend our knowledge of the Moroccan diversity of *Dactylogyrus*, molecular characterization was carried out using mitochondrial (COI) and nuclear markers (18S, ITS-1 and 28S). This study is the first to apply a barcoding approach to Moroccan species of *Dactylogyrus*. Barcodes were obtained from 20 specimens belonging to four species of *Dactylogyrus*, and intraspecific divergence was noticed among the COI sequences. **Significance:** The present study highlights the importance of the use of the barcoding approach to elucidate the taxonomic status of *Dactylogyrus* infecting cyprinid fishes.

Are mini-DNA barcodes sufficiently informative to resolve species identities?

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Background: DNA barcoding has become a popular diagnostic tool to assign species-specific signatures. In plants, a number of chloroplast DNA regions such as *psbA-trnH* have been shown to successfully discriminate members of various taxa. However, the technique is not always successful, as in the case of museum specimens or samples used in the raw drug trade, where the DNA is often degraded. In this context, recent studies have suggested the use of shorter stretches of the region, called mini-barcodes, to resolve species identity. The mini-barcodes are relatively more stable and easily recovered from the degraded DNA. In this study, an attempt was made to compare the effectiveness of mini-barcodes over full-length DNA barcodes in differentiating 16 species of *Phyllanthus* (Phyllanthaceae) used in the herbal trade. Using an in silico approach, mini-barcodes of varying lengths (50–200 bp) of the region *psbA-trnH* were generated and evaluated for their ability to resolve the 16 species in comparison to the full-length DNA barcode (398 bp). **Results:** The mean inter- and intraspecific divergence using the full-length DNA barcode of these species was 14.92% and 0.48%, respectively. In contrast, the mean interspecific divergence obtained from the various mini-barcodes ranged from 8.87% to 30%. The mean percent species resolved increased with the length of the barcode. Only certain mini-barcodes such as 150 and 200 bp could resolve 100% of the species. These results were also reflected in the correlation between the p-distances among various mini- and full-length barcodes. **Significance:** In summary, the results indicate that while mini-barcodes may resolve species identities as much as is accomplished by full-length barcodes, it is also associated with a high degree of variance. Hence, due caution may have to be exercised while using mini-barcodes to unravel species identities pertaining to museum samples or those used in herbal trade.

Thermal adaptation as the first stage of parapatric speciation in coastal South Africa

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Numerous marine species that occur along the South African coast can be divided into cryptic species that are often only identifiable using DNA barcoding. In most cases, the ranges of these species coincide with the boundaries between the region's temperature-defined marine provinces. This suggests that differences in water temperature may be the main drivers of incipient speciation. We provide examples

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of coastal species in which there is as yet no genetic differentiation on the basis of COI sequences, but in which certain temperature-selected genes already show differentiation that matches the boundaries between marine provinces evident in co-distributed species. We discuss the implications of selectively neutral barcoding markers for the management of marine resources.

Who am I? DNA barcoding of the mouse lemur occurring in the Sahamalaza National Park

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DNA barcoding is being widely used as a research tool for refining the understanding of biodiversity. This approach is relevant for a biodiversity hotspot country like Madagascar, with more than 90% endemics such as the lemurs group. With a complex hydrogeographic system, the northern part of Madagascar harbors highly diverse mouse lemur (*Microcebus*) populations. Whenever *Microcebus* populations are discovered, their taxonomic identification relies on the combination of morphometric measurements, photographic material, and the post-hoc application of DNA barcoding that is performed in comparison to sequences from known species. We discover a *Microcebus* population from Sahamalaza peninsula Anabohazo forest, which is in need of identification via DNA barcoding. A 931-bp length of cytB fragment was generated for two individuals from tissue samples. Newly generated sequences were aligned with 125 sequences that stemmed from 18–21 described species of *Microcebus* with MEGA 6. Species identity was established by constructing a neighbour-joining tree with 1000 bootstrap replicates based on the number of pairwise differences. Our two sequences differ from Cyt b haplotype of *M. sambiranensis* (RMR38, RMR39, RMR40 from GenBank) with only 4 bp. Yet, this haplotype differed from all other mouse lemur taxa by an average of 86 bp (min. = 30 bp, max. = 128 bp). Given this high genetic similarity, it can therefore be concluded that the two individuals belonged to this species. The mouse lemur population is subsequently assigned to *M. sambiranensis*. Globally, 94% of lemur species are threatened with extinction. Discovering new populations, such as we found in Anabohazo forest, Sahamalaza Peninsula, is very important on the one hand to establish conservation priorities and on the other to promote the area itself for ecotourism or other research activities for the sustainable use of the natural resources. DNA barcoding provides an effective tool to identify populations at species level, especially for populations with high morphological similarities such mouse lemurs.

The quality control conundrum: using DNA barcoding and chemical profiling for authenticating species of *Pelargonium* used in commercial herbal products

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Background: Several species of *Pelargonium* are indigenous to South Africa and are highly valued for their horticultural value and medicinal properties. For hundreds of years various ethnic groups have used root extracts of *P. sidoides* as a remedy to treat coughs, upper respiratory tract irritations, and gastrointestinal conditions. An ethanolic extract is used in the proprietary herbal tincture known as umkaloabo that is currently successfully marketed in Germany with sales that have escalated over 700%. The use of commercial herbal products has surged globally in recent years, and as a result the phytomedicine industry is under immense pressure to develop rapid, accurate, and economical methods for quality control, especially for the positive identification of raw materials. The taxonomic delimitation of the two taxonomic allies *P. sidoides* and *P. reniforme* has been debated in literature. Most consumer products explicitly refer to the botanical active as *P. sidoides*, posing quality control concerns as *P. sidoides* and *P. reniforme* have not been proven to be pharmacologically equipotent. **Results:** Here, we used DNA sequence information generated through standardized DNA barcoding techniques for the authentication of *P. sidoides* products. A more comprehensive molecular phylogeny for the section *Reniformia* within the Geraniaceae was investigated. Furthermore, a DNA barcode reference library for the section *Reniformia* was added to the Barcode of Life Data System, and several herbal medicines tested showed not to contain DNA material of *Pelargonium*, indicating potential adulteration of the said products. **Significance:** This is the first attempt to compile a reference library of DNA barcodes for herbal medicines in South Africa, which will provide species-level identification for herbal medicines traded in the country. The reference sequences generated in the project were used to effectively compare against sequence data of commercial herbal products, and adulterated herbal products were identified.

Species admixtures in herbal trade: causes, consequences, and mitigation

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The global economy of the international trade of herbal products has been increasing by 15% annually. Most of the raw materials for the herbal products are sourced from south and south-east Asian countries. India along with China are the major suppliers of raw herbal drugs. In India, around 960 species are in active trade, and most of these are sourced from the wild. Many of these plant species are in short supply due to the lack of cultivation of the species or rarity of the species in the wild. However, with increasing international trade in herbal medicinal products, and due to a paucity of the material, there is an increasing concern about the widespread adulteration and species admixtures in raw herbal trade. The adverse consequences of such species admixtures on the health and safety of consumers are only recently beginning to be recognised and documented. We have assessed the extent of adulteration in raw herbal trade of a number of important medicinal plants in South India using DNA barcoding. We discuss the nature and magnitude of species adulteration in raw herbal trade. Besides, we also discuss the possible biological and chemical equivalence of the species admixtures and substitutes and their consequences thereof to consumer health and safety. Finally, a framework for the development of an herbal trade authentication service that can help regulate the herbal trade market is proposed.

Amphibian's inventory in Marojejy National Park (Madagascar) with DNA barcoding identification

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Background: Madagascar, classified as a microcontinent that has been isolated since the Cretaceous, is an island rich in biodiversity. Its fauna and flora represent a high endemicity rate and are at the greatest risk. This global hotspot has become an interesting background for testing hypotheses and understanding issues surrounding nature. An inventory was made in the Marojejy National Park, a mountain range from 80 to 2133 m above sea level in the northeast of the island. This

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study was aimed at understanding the altitudinal distribution and the ecological preference of amphibians. DNA barcoding is one of the methods used in species identification. **Results:** During the field study, 41 species of Amphibia were inventoried. There are more species located between 800 and 1300 m altitude. In total, 210 tissue samples were collected and studied in the laboratory. Furthermore, through genetic identification, 128 of the samples did not share the same names of the species assumed during the field study. The number of species identified increased to 46. **Significance:** There are preferable ecologies for each species; there are the "ubiquists" that are found at each altitudinal stage and "specialists" who have preferential altitudinal stages. Inventories may be carried out through the visual identification method, though a genetic method provides more precision and certainty. DNA barcoding is a precision method, which can be used for the inventory of biodiversity and taxonomy.

DNA barcoding of *Mistichthys luzonensis* Smith, 1902 (Percifornes: Gobiidae), the world's smallest commercial fish

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Background: The Philippine endemic freshwater goby, *Mistichthys luzonensis* Smith 1902, is considered the smallest commercial fish. The species is restricted to lakes Buhi, Bato, Katugday, and Manapao, and the Bicol river system in Camarines Sur in the Philippines. The species used to be abundant in lakes Bato and Buhi, but it collapsed in the 1970s due to the use of motorized pushnets, over-exploitation, and the introduction of tilapia into the lakes. Since then, the populations in both lakes have not recovered. Another small goby endemic to the Philippines, *Gobiopterus lacustris* Herre 1927, also commercially important, looks very similar to *M. luzonensis*. The two species are commonly misidentified. **Results:** Ten specimens of *M. luzonensis* were DNA barcoded using the mitochondrial cytochrome *c* oxidase I (COI) from each of two lakes—Lake Bato and Lake Manapao. Despite repeated sampling, no samples were collected from Lake Buhi and Lake Katugday. Ten specimens of *G. lacustris* collected from Laguna de Bay were also DNA barcoded. A total of 37 COI sequences were analyzed, including five *G. lacustris* sequences and two *G. semivestitus* downloaded from GenBank. The neighbour-joining tree constructed using the Kimura 2-parameter (K2P) model showed a single cluster for *M. luzonensis* with 99% bootstrap support, and two sub-clusters: all 10 specimens from Lake Bato formed one sub-cluster, while the 10 specimens from Lake Manapao formed a second sub-cluster. Twelve out of the 15 *G. lacustris* specimens formed a single cluster, which joined with the *M. luzonensis* cluster. The other three specimens formed a separate cluster. The average intraspecific K2P genetic distance of *G. lacustris* was 8.4% while the interspecific distance between *G. lacustris* and *M. luzonensis* was 7.7%. **Significance:** Although morphologically alike, the two species can be discriminated from each other using DNA barcoding.

Barcode Index Numbers expedite quarantine inspections and aid the interception of nonindigenous mealybugs (Pseudococcidae)

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Quarantine interception of invasive and nonindigenous insect pests at ports of entry is often impeded by the lack of robust identification methods. Due to their inconspicuous morphology and wax-covered bodies, mealybugs present a particular challenge. The present study employs DNA barcoding (658 base pairs from 5′-end of cytochrome *c* oxidase I) as a tool for discriminating between species of mealybugs because of its proven utility in discriminating closely related taxa and clarifying their distributions. The current study considers DNA barcodes from 914 mealybugs (Pseudococcidae) collected in 31 countries. Most (836) sequences derived from specimens that were assigned to a named species, but the others were only identified to a genus or family. The Barcode Index Number (BIN) system assigned these 914 sequences to 120 BINs, nearly doubling the putative species count. With a single exception, intraspecific divergence values for named species were less than their nearest-neighbour (NN) distance. However, 13 species showed BIN splits, and two species were merged in a BIN. The analysis displayed a high mitochondrial diversity in Pseudococcidae with confamilial distances up to 27%, and revealing cases of potential cryptic species or misidentifications. The study affirms the utility of BINs for the rapid recognition of nonindigenous insect pests at quarantine stations.

Hidden no more: metabarcoding reveals patterns and correlates of soil microbial diversity across Amazonia

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The quantification of biological diversity is crucial for biogeography, ecosystem services, biological interactions, and conservation. Metabarcoding offers a quick and highly accurate alternative to classical taxonomical surveys for quantifying biodiversity. Even in highly diverse and poorly sampled environments such as tropical rainforests—where sequence reference databases are very thinly populated—the use of operational taxonomic units (OTUs) defined on the basis of molecular variation makes the assessment of diversity across sites possible. Here, we use metabarcoding to analyse the total prokaryote and eukaryote diversity in environmental samples (soil and litter) from 39 survey plots in a longitudinal transect across the Brazilian part of the Amazon rainforest, using the 16S and 18S markers, respectively. We use these data to characterize alpha diversity and community composition based on OTUs, and to test hypotheses on their correlation with longitude and different habitat types and soil characteristics. We find that OTU richness of 16S and 18S are weakly correlated and differ significantly among localities and habitats. Our results (*i*) provide a first large-scale mapping of Amazonian soil diversity, suggesting that OTU soil patterns, mostly dominated by microorganisms, might follow substantially different patterns than observed for mammals, trees, and birds; and (*ii*) indicate that multiple environmental factors interact in determining soil OTU richness patterns and community composition.

Fish DNA barcoding around a marine mega-infrastructure to improve environmental assessment and monitoring

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Background: Accurate species identification plays a pivotal role in environmental monitoring. Species-level assessments and monitoring provide the required resolution to estimate biodiversity parameters. The terminal port of PERU LNG is a large infrastructure located on the central coast of Peru. This infrastructure has increased the fish species richness, including taxonomically challenging groups. This is particularly true for species belonging to the family Sciaenidae. Since challenges to correctly identify all fish species continue, limiting our knowledge of biodiversity, we implemented an integrative approach for improved species identification. We conducted a DNA barcoding

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study as this approach and prior studies have demonstrated that accurate fish identification can be achieved by using molecular techniques. **Results:** We constructed a DNA barcode reference library that can be applied around our study area and in similar nearby habitats. We collected and analyzed 56 vouchered specimens and identified specimens using morphological characteristics. We identified 24 unique species belonging to 24 genera, seven families, and eight orders. The intraspecific divergence ranged between 0% and 0.64%, and interspecific divergence ranged between 10.8% and 33.6%. Automatic Barcode Gap Discovery (ABGD) analysis discarded the presence of cryptic species in our study area. A local BLAST between our reference library and samples from the same locality and season, but without taxonomic validation, resulted in 19 matches (65.5%) with high identity values. For specimens of the family Sciaenidae we confirmed the paraphyly of the genus *Stellifer* and clade homogeneity in the genus *Menticirrhus*, suggesting that problems in identification may have an explanation outside of evolutionary history. **Significance:** We highlight the importance to implement DNA barcoding for complementing biodiversity assessments in marine environments. Although the present study developed a comprehensive DNA barcode library at local scale, it represents the first step in generating a larger DNA barcode reference library for marine fishes in Peru and the Humboldt Current Large Marine Ecosystem.

Diversity patterns revealed by DNA barcodes: pan-Arctic variation in the arthropod communities visiting flowers of the genus *Dryas*

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Background: Pollination is an ecosystem function of global importance. However, who visits the flower of specific plants, how the composition of visitors varies in space and in time, and how such variation translates into pollination services is typically hard to establish. Given their enormous diversity, flower-visiting arthropods are simply hard to tally, let alone to describe in terms of other aspects of diversity. To clarify regional variation in the pollinator community of a circumpolar flower resource, we compared the structure of the arthropod community pollinating *Dryas* spp. across 14 sites of the Northern hemisphere. In a distributed experiment, pollinators were sampled with 100 sticky flower mimics at each site. At one site in north-east Greenland, spatiotemporal resolution was added by replicated sampling at 15 locations within a valley. All insects caught were identified to species level using a partial sequence of the mitochondrial COI gene, and diversity patterns described by both Barcode Index Number (BIN)-level richness and by phylogenetic estimates of diversity. **Results:** Across the Arctic, we sampled a total of 13 826 arthropods visiting sticky *Dryas* mimics. Of these arthropods, we successfully sequenced and identified 11 229 individuals, detecting a total of 1288 different BINs. At the time of writing, we are frantically comparing patterns of species turnover, phylogenetic diversity, and BIN richness across the Arctic, with results to be reported at the conference in November. **Significance:** DNA barcodes allow us to overcome the taxonomic impediment, and to address ecological patterns involving thousands of taxa, each of which are hard to identify. DNA barcodes also contain (some) information on species relatedness, thus allowing us to simultaneously assess how phylogenetically diverse communities are formed on a single plant resource under different biogeographic and abiotic conditions. Taken together, this information offers unparalleled insights into community assembly processes in space and in time, with direct implications for ecosystem functioning.

Studying hyperdiverse lepidopteran communities in French Guiana with DNA barcoding

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Background: French Guiana is a French Overseas Department of ~84 000 km², 98% being covered by dense, overwhelmingly primary, equatorial Amazonian forest. Terrestrial habitats belong to the Guiana Shield biogeographical region, hosting an outstanding, largely unexplored invertebrate diversity. **Results:** Since 2010, our inventory of lepidopteran communities of several sites has implemented an integrative approach where DNA barcoding is the initial screening tool for specimen curation followed by diversity analyses. The thousands of records generated by these surveys have been complemented in BOLD (the Barcode of Life Data System) by independent projects focusing on the taxonomy of several families, for a current total of nearly 20 000 records representing more than 5000 Barcode Index Numbers (BINs). Here, we present a summary of the current coverage of this regional DNA barcoding library, and we emphasize through examples how it significantly accelerates species discovery and description and how it improves our understanding of spatial and temporal turnover in lepidopteran communities. **Significance:** The massive DNA barcoded reference collection assembled at Museum national d'Histoire Naturelle is a fundamental resource for biologists working on the diversity of these insects in Amazonia.

Plant DNA barcoding: a decade of success and failure

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Background: Our tryst with plant DNA barcoding started in 2007. We worked with zeal but could not impress our sponsors due to the unusual results: Barcode loci could not resolve species identity. Out of the 17 species analysed, only one species could be identified. Morphological, geographical, and molecular data analyses suggested probable reticulate evolution, and thus barcode markers may not work in this case. Most of the other plant projects supported by DBT at the time were not very encouraging, as far as species identification was concerned. This was mainly due to, among other reasons, selection of complex genera in most of the projects. But we continued our efforts with in-house project support and partly with Department of Science and Technology. **Results:** Using 300 accessions of tree species collected from our botanical garden and other parts of India, mostly Uttar Pradesh, we tested the efficacy of standard plant barcode loci. The species discrimination ability of ITS ranged from 24.4% to 74.3% and that of *trnH-psbA* was from 25.6% to 67.7%, depending upon the data set and the method used. Species resolution by ITS2 and *rbcL* ranged from 9.0% to 48.7% and from 13.2% to 43.6%, respectively. During 2012, we planned for a network project comprising different laboratories of CSIR, New Delhi, to build up a plant barcode consortium supported by CSIR. Under this project, we attempted to investigate many species but could only analyze around 40 medicinal plant species and a few lichen species from a biodiversity-rich national sanctuary. The reasons for failure were many, including funding and lack of

coherence among laboratories. **Significance:** Our constant efforts will continue to see the completion of the project. CBOL's support was the key to the morale boosting in continuing our activities.

Using taxonomic consistency with semi-automated data pre-processing for high quality DNA barcodes

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Background: In recent years, large-scale DNA barcoding campaigns have generated an enormous amount of DNA barcodes, which are usually stored either in NCBI's GenBank or the official Barcode of Life Data System (BOLD). In the course of the initiative, German Barcode of Life (GBOL), data were generated for the reference library of 2850 species of Coleoptera from 13 516 individuals. **Results:** Confronted with the high effort associated with the identification, verification, and data validation, a bioinformatic pipeline in R, TaxCI was developed that (*i*) identifies taxonomic inconsistencies in a given tree topology (optionally including a reference data set), (*ii*) discriminates between different cases of incongruence in order to identify contamination or misidentified specimens, and (*iii*) graphically marks those cases in the tree, which finally can be checked again and, if needed, corrected or removed from the dataset. For this, TaxCI uses either DNA-based species delimitations from other approaches (e.g., mPTP) or performs an implemented threshold-based clustering. The data-processing pipeline, including the newly generated set of barcodes, was tested using previously published barcodes of beetles occurring in Germany as reference dataset. A data revision based on the first run of the TaxCI tool resulted in the second TaxCI analysis in a taxonomic match ratio very similar to the one recorded from the reference set (92% vs. 94%). The latter improved by nearly 20% through this procedure. **Significance:** Overall, the new evaluation pipeline for DNA barcode data allows for the rapid and easy identification of inconsistencies in large datasets, which can be dealt with before submitting them to final data repositories like BOLD or GenBank. Ultimately, this will increase the quality of submitted data and the speed of data submission, while primarily avoiding the deterioration of the performance of the data repositories due to ambiguously identified or contaminated specimens.

Evaluation of multilocus marker efficacy for delineating mangrove species of west coast India

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Background: Plant DNA barcoding is complex and requires more than one marker compared to animal barcoding. Mangroves are diverse estuarine ecosystems prevalent at tropical and subtropical zones, but anthropogenic activity turned them into vulnerable ecosystems. For conservation strategies, there is a need to build a molecular reference library based on molecular markers along with morphological characteristics. **Results:** In this study, we tested the core plant barcode (*rbcL* + *matK*) and four promising complementary barcodes (ITS2, *psbK-psbI*, *rpoC1*, and *atpF-atpH*) in 14 representative mangrove species belonging to five families from the west coast of India. Data analysis was performed based on barcode gap analysis, intra- and interspecific genetic distance, Automated Barcode Gap Discovery (ABGD), TaxonDNA (BM, BCM), Poisson Tree Processes (PTP), and General Mixed Yule-coalescent (GMYC). The *rbcL* locus showed highest PCR efficiency and sequencing success (100%) rate, followed by ITS2 and *matK*. Using a single locus for analysis, ITS2 exhibited the highest discriminatory power (87.82%) but combinations of *matK* + ITS2 provided the highest discrimination success (89.74%) rate except within the genus *Avicennia*. The single ITS2 barcode locus resolved *Rhizophora apiculata* and *R. mucronata* based on GMYC analysis, and species of *Sonneratia* were demarcated using ABGD tools with relative gap width (*X*=1.5). Furthermore, we evaluated three additional markers (*psbK-psbI*, *rpoC1*, and *atpF-atpH*) for species of the genus *Avicennia* (*A. alba*, *A. officinalis*, and *A. marina*), of which the *atpF-atpH* locus was able to discriminate three species of *Avicennia* based on ABGD and TaxonDNA analysis. **Significance:** Our analysis underscored the efficacy of *matK* + ITS2 markers with *atpF-atpH* as the best combination for mangrove species identification in the west coast region of India.

Use of environmental DNA metabarcoding for fish biodiversity assessment in Neotropical rivers

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Background: The management and conservation of species relies on accurate species identification and reliable information on spatio-temporal distribution and habitat use. Due to difficulties of traditional taxonomic identification and monitoring techniques, environmental DNA (eDNA) has recently exploded as a promising tool for biodiversity assessment. Here, we applied amplicon-based Illumina sequencing to characterise fish biodiversity along most of the main stem of the Jequitinhonha River (southeast Brazil). Filtered water (6 L preserved in molecular grade ethanol, silica beads, and surfactant benzalkonium chloride) and sediment samples were obtained from 11 sample sites. Amplifications were conducted using partial sequences of the mitochondrial genes COI (340 bp) and 12S (106 bp). **Results:** Illumina MiSeq analysis yielded 6.5 million reads and allowed the detection of the known biodiversity of the river, including introduced species (e.g., *Prochilodus argenteus*, *Astronotus ocellatus*). Sediment samples yielded a greater number of eDNA copies compared to water samples, and filters preserved in silica beads provided better results than the ones preserved in ethanol. Some spatial differences among sections of the river could be detected, and they were interpreted on the basis of habitat type and anthropogenic impact. Universal COI primers provided a more reliable identification and distinction of closely related species when compared to the ribosomal gene 12S; however, due to the universality of the COI primers, we obtained a vast amount of micro-eukaryotic reads (95%) and only 5% of vertebrate reads. **Significance:** These results demonstrated that eDNA can contribute to fish biodiversity assessment in Brazilian basins and highlights the issues pertaining to the choice of genetic markers for metabarcoding.

Synergies in national biodiversity campaigns: cooperation adds quality to species knowledge bases

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The Norwegian Barcode of Life (NorBOL) is a network of biodiversity institutions and scientists engaged in DNA barcoding of the fauna and flora in Norway. The network is an integrated part of the global Barcode of Life Initiative with a vision to assemble a comprehensive reference library for research and management of biodiversity in Norway and Polar regions. The Norwegian Biodiversity Information Centre is an official national source for biodiversity information in Norway. The Centre coordinates the Norwegian Taxonomy Initiative (NTI), established by the Ministry of Climate and Environment in 2009. The pri-

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mary objective of NTI is to strengthen the knowledge of Norwegian biodiversity and to stimulate recruitment and education of the next generation of taxonomists. NorBOL and NTI have had a very fruitful cooperation since 2009. Through NTI-supported inventories, a large amount of voucher material of Norwegian species, identified by taxonomic experts, are available for DNA barcoding. Annual barcode workshops for project managers and other scientists involved in DNA barcoding ensure capacity building and expertise. Joint efforts also contribute to better accessibility and dissemination of biodiversity data in Norway and ensure that data on names and taxonomy, geographical distributions, DNA barcodes, etc. are made available through many public infrastructures and services. Access to correct species information is fundamental for biodiversity research and management, and the quality and accessibility of these data from Norway has increased through the synergy between NorBOL and NTI.

The Norwegian Taxonomy Initiative

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Experts have assumed that one in four species remains to be discovered in Norway. The Norwegian Taxonomy Initiative (NTI) helps to fill these knowledge gaps by funding inventories to map and identify poorly known groups of multicellular species in the country. Biodiversity data from the inventories are made easily accessible to society through a range of infrastructures and services. Inventories on a variety of taxa are carried out throughout Norway's' diverse habitats; from wetlands in the arctic north to dry and warm areas in the south, from mountains to lowlands, from streams and lakes, and from littoral to deep waters. So far, nearly 2400 species new to the country have been discovered, of which 30% are new to science. And there is a lot more to be discovered.

The genomic substrate for adaptive radiation in Lake Tanganyika cichlid fishes

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The Great Lakes of East Africa are, collectively, the earth's most remarkable and species-rich freshwater feature. Much of the lakes' spectacular organismal diversity evolved through adaptive radiation and explosive speciation within a timeframe of a few millions to tens of thousands of years only. For example, hundreds of endemic species of cichlid fishes have evolved independently in each of the three Great Lakes Victoria, Malawi, and Tanganyika, making these species flocks the taxonomically and phenotypically most diverse ongoing adaptive radiations in vertebrates and important model systems in evolutionary biology. We have sequenced the genomes of virtually all cichlid species in the oldest of the three lakes, Lake Tanganyika, in order to shed light on the genomic underpinnings of adaptive radiation and explosive speciation in cichlid fishes.

Integrative taxonomy of the crinoids (Echinodermata: Crinoidea) of the shallow waters of KwaZulu-Natal, South Africa

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Background: Marine biodiversity of eastern Africa is relatively poorly known, with great disparities in taxonomic and geographical coverage and large gaps in taxonomic data. The coastline of KwaZulu-Natal in the south-east has been reasonably well explored for echinoderms, resulting in a number of recent taxonomic revisions for the region. However, the last comprehensive taxonomic revision for Crinoidea dates from 1976. An urgent update of the fauna was thus needed. Given the intricate morphological characters of crinoids, DNA barcoding was used to delimitate taxa. **Results:** Five recent expeditions to the shallow-waters (50 m depth max.) of KwaZulu-Natal (1999–2016) resulted in a modern voucher collection of echinoderms. The bulk of this material is deposited in the Royal Museum of Central Africa in Tervuren, Belgium. This study focuses on the collected crinoids. DNA barcodes were generated for most of the specimens (96 of 113 samples or 85%). Independent morphological examination led to species identification. The number of shallow-water crinoids of KwaZulu-Natal was raised from 5 to a putative number of 10 species. All appear to be typical tropical species. It is interesting to note that barcode fragments were successfully obtained from ethanol preserved, but also from dried, specimens, giving promise that other museum collections, which often store their crinoids dry, can also be harvested to expand the BOLD library. **Significance:** Given that marine biodiversity is an important source of income for South Africa, either directly through resource exploitation or indirectly through ecotourism or through ecosystem services, it is of paramount importance that marine biodiversity is properly documented and understood. Barcoding helps in this endeavour. These are the first DNA barcodes of crinoids for eastern South Africa.

DNA barcoding echinoderms of the east coast of South Africa

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Background: According to the Barcode of Life Data System (BOLD, http://www.boldsystems.org), approximately a fourth of the described echinoderm species have been already barcoded (more than 2000 species barcoded out of the about 8000 species described). However, only fewer than 300 of the ~29 000 echinoderm barcode records available on BOLD are from South Africa, a country with a coastline of more than 2500 km on both the Atlantic and Indian Oceans. In an effort to explore the echinoderm diversity of South Africa, we barcoded 351 specimens collected during five different campaigns (from 1999 to 2016) in the North and South of the KwaZulu-Natal Province, across two distinct offshore environments. **Results:** Cross comparison between morphological and molecular identification allowed distinguishing ~114 species, including a number of new records for the country and some putative new species. These included Crinoidea (96 specimens and ~10 species), Ophiuroidea (95 specimens and ~44 species), Asteroidea (48 specimens and ~19 species), Echinoidea (27 specimens and ~12 species), and Holothuroidea (85 specimens and ~29 species). Nonetheless, DNA barcoding revealed unexpected large intraspecific distances (suggesting additional overlooked species) as well as clusters of heterospecific sequences (suggesting either poor marker resolution or the need for further taxonomical consideration). DNA barcodes obtained for more than 40 specimens showed distances of more than 1% with the DNA barcodes currently available in BOLD and GenBank. **Significance:** This data set will be further investigated using integrative taxonomy and will deliver a valuable addition to the reference library of DNA barcodes for echinoderms.

Closed-Tube DNA Barcoding of fish species and subspecies in a laboratory or on location using one set of reagents

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Background: Species mislabeling of fish products costs billions of dollars in the US market alone, threatens sustainability of fish stocks worldwide, and increases the risk that pathogens go undetected. FDA protocols use conventional PCR amplification of the COI barcoding tar-

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get, followed by sequencing. Testing in regional laboratories is costly, complex, and relatively slow. Consequently, only a tiny fraction of fish products are analyzed. Closed-Tube Barcoding of edible fish is convenient, fast, low-cost, and reliable. An extremely small sample is mixed with a lysis reagent, diluted, and added to a universal PCR master-mix containing FDA-approved primers. Closed-Tube Barcoding uses LATE-PCR to generate single-stranded COI targets. These targets are coated at end-point with sets of Lights-On/Lights-Off probes. When the probes melt off their target, a species-specific fluorescent signature is generated. **Results:** We compared ≥7000 sequences to identify two segments within COI that are sufficiently variable among edible fish to generate two fluorescent signatures using differently colored probes. The resulting 2D-fluorescent signatures are highly specific. Archival samples of verified species are being analyzed to construct a library of fluorescent signatures for >700 species and subspecies. Standard real-time PCR machines, as well as portable devices, are being compared. Each PCR product tested is also sequenced. Thereafter, only novel signatures need be sequenced once. All data will be linked to their corresponding entries in the FISH-BOL database. Thereby, an expanding library of fluorescent signatures will become a resource for rapid species authentication. **Significance:** Closed-Tube DNA Barcoding will reduce the cost and time to detect seafood fraud anywhere in the supply chain. Closed-Tube Barcoding can also be used for analysis of virtually any group of animals, plants, or microbes on earth by designing appropriate primers and probe sets covering numerous genera and species. Funded by Brandeis University and the National Fisheries Institute.

Authentication of herbal plants and products using DNA-based biological reference material library

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Background: Herbal medicinal products have become of global importance, for health benefits and economic considerations. India is considered the "medicinal garden" of the world, with 8000 medicinal plants, of which 960 are commercial species that are traded nationally and globally. India does not have any published marketplace studies and subsequent estimates of adulteration in an industry facing considerable supply demands. Hence, the objective of this study is to develop a DNA-based Biological Reference Material (BRM) library for Indian herbal plant and products. **Results:** The library consisted of 187 vouchered herbal species. About 93 herbal products were authenticated using the DNA barcode regions *rbcL* and ITS2, which showed 40% of the products tested are authentic and 60% of the products are adulterated (i.e., contained species not listed on the product labels). The adulterated samples included contamination (50%), substitution (10%), and fillers (6%). The tested herbal plants covered 76 species (45 families) and 23 different types of sample materials in the form of fresh, dried, extract, and powdered substances. A standard experimental protocol (EP) was used to test all the samples. Among the tested samples, nearly 53% of the samples from 35 families that covered 17 different types of sample materials were validated with accurate identification. **Significance:** The development of vouchered, curated DNA-based BRM libraries and authentication using DNA barcoding will provide a competitive advantage to herbal industries in manufacturing an authentic, high-quality product, thereby increasing consumer confidence and preference.

DNA barcoding reveals the medicinal value of honey by its floral composition

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Background: Honey is a natural product highly consumed due to its known health benefits. It has been reported to show inhibitory effects on around 60 species that include bacteria, fungi, and viruses, and it serves as a remedy for burns, ulcers, wound healing, etc. Medicinal properties of honey are determined by floral origins. Methods for identification involve palynological analysis, chromatographic methods, and direct observations of bee behavior. However, these methods can be less sensitive and time consuming. Hence, in the present study, DNA barcoding was used for plant species identification by using pollen DNA. For this, 36 honey samples were collected from five different districts of Mizoram, North East India. Pollen grains were isolated, genomic DNA was extracted for PCR amplification using ITS2 and *rbcLa* primer sets, and sequences were used to identify plant species. So, it is very clear that DNA barcoding is fast, easier to actualize than classical methods, and is suitable for studying plant diversity and the topographical origin of honey. **Results:** In this study, DNA barcoding analysis of honey samples revealed the species, *Macaranga indica* and *Mikania micrantha*, that are used to treat the venereal sores and syphilis, and it also proved the habitat to be typical deciduous forest. However, in samples collected at Mamit, *Combretum indicum* was identified, which is used as astringent and anthelmintic. These natural flavonoids are believed to be present in the honey samples. **Significance:** The ease of administration is an important characteristic for the use of honey as a remedy for treatments. Therefore, pollen molecular characterization using DNA barcoding have been proved to be very useful for the authentication of socio-economically important honey product. However, there is a mounting market for honey as a health product, with recent research proving the potential health benefits as a medicinal product.

Bridging biodiversity evidence through data standards: the GBIF perspectives towards molecular data

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Global Biodiversity Information Facility (GBIF) has grown to accommodate evidence from many sources, including citizen science and quantitative ecology. A critical requirement is to expand this network to accommodate evidence from molecular research. GBIF.org aims for universality in its data discovery services, supporting integration, search and filtering capabilities, documenting data provenance, and promoting best practice around data citation. By early 2017, the GBIF network includes several datasets of molecular origin. These early efforts require further enhancements around data linkage and attribution, particularly through making connections between specimen data and associated molecular information. GBIF's ambition is to accelerate processing of all data records to cluster related data records derived from specimens, sequences, publications, and other sources. GBIF and Barcode of Life Data System (BOLD) need to establish a continuous feed for new sequence data to be incorporated within GBIF. As the barcode of life community continues to expand, growing volumes of data will flow from field-based monitoring activities that rely on barcodes to determine the set of taxa recorded. The interpretation of the growing volumes of sequences will evolve as reference libraries improve. These data will serve as one of the key streams of evidence for species distribution. GBIF aims to work closely together with molecular infrastructures to (*i*) form cross-linkages between digitized specimens and associated barcode data, (*ii*) to accommodate spatio-temporal data from environmental sequencing projects, and (*iii*) to expand the current taxonomic backbone to include operational taxonomic units based on molecular and other evidence, including BOLD Barcode Index Numbers (BINs). Further, GBIF could support organisation and visualisation of data on infraspecific genetic variation as part of the representation of species distribution data.

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Closed-Tube DNA Barcoding analysis of the species and global distribution of *Naegleria*: a worldwide genus of single-celled amoeboflagellates

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Background: *Naegleria* are abundant, free-living, freshwater amoebae with a worldwide distribution that are able to differentiate into swimming flagellates. Species within this genus are quite diverse, probably because the genus evolved over a billion years ago. Mesophilic species like *N. gruberi* are found on the five temperate continents, while *N. antarctica* are thermophobic, and *N. fowleri* are thermophilic opportunistic human pathogens (the brain-eating amoebae). Previous analysis of short ribosomal ITS sequences catalogued roughly 40 geographically separated species. Our analysis, using a combination of Closed-Tube Barcoding of the COI gene target plus DNA sequencing, has improved the definition of species. Analysis of about 75 clonal isolates from around the world using both COI and ITS sequences show agreement in the degree of relatedness among isolates in most cases. **Results:** Closed-Tube DNA Barcoding is an efficient, cost-effective method for amplifying the COI barcoding target sequence from large numbers of isolates and then scanning the resulting single-stranded DNA for sequence variations using Lights-On/Lights-Off probes. In order to resolve all species within this genus, we use a universal set of nine probes having three subsets. Subsets are labeled in different fluorescent colors. This experimental design allows us to compare the fluorescent signatures of different isolates and immediately observe whether sequence differences are clustered in one region of the COI target, or distributed throughout. These predictions can be confirmed by using Dilute-N-Go sequencing. **Significance:** Our ground-breaking study of *Naegleria* provides an affordable method for characterization of species and species variation within microscopic eukaryotes—a world that is largely unknown. Using this approach we will be able to map species distributions in small or large ecosystems. We can also use our approach to selectively test for *N. fowleri*, or virtually any pathogen in a water sample. Supported by Brandeis University.

Two for one: using field expeditions for inventories and evolutionary analysis

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Traditionally, field expeditions as organised by, e.g., natural history museums serve an inventory goal: specimens are collected and identified; new records and new species are published. The specimens are deposited in institutional collections, and the results are often published in a taxonomic monograph. These days, DNA barcoding is sometimes added to the routine work flow, with the added benefit of easier species delimitation and a richer data output per specimen. However, if properly planned, the inclusion of DNA barcoding also enables traditional expeditions to address novel evolutionary questions in addition to their more traditional, inventorying aim. As an example, I will introduce the large bi-national expedition to the hotspot of tropical endemism Gunung Kinabalu, in Malaysian Borneo, which allowed us to answer questions pertaining to the origins of the endemic biota on this young, isolated mountain. Using DNA barcodes for selected groups of plant, animal, and fungal taxa, including Kinabalu-endemics as well as widespread sister species, we were able to determine the two major evolutionary routes by which the Kinabalu endemic biota originated. At present, we are exploring ways to apply similar approaches to determine the origins of the endemic biotas of other isolated regions in Borneo.

Molecular Weevil Identification project with a novel molecular–taxonomic approach to close the barcode gap

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Background: Barcoding projects often reveal cryptic species or synonyms. These "side results" often do not lead to taxonomic changes, because it is often impossible to estimate a reliable barcoding gap, especially if just a single sequence steps out of line. **Results:** The objective of the Molecular Weevil Identification (M.W.I.) project was to build a comprehensive linked library of mounted reference collection, tissue collection (Biobank), genetic reference samples (DNA), and *COI* barcode data. Over 5000 specimens of mostly European weevils have been processed, comprising ~25% of the Western Palearctic Curculionidae fauna. During the project, ~50 new species have been described. To make future taxonomic evaluation possible, a new combined molecular–taxonomic approach has been developed to set reliable barcode gaps for all major genera. **Significance:** Reliable barcode gap data are often requested, but never provided, neither from the molecular or bioinformatics, nor from the taxonomy side. On mathematical methods, ecological data are not taken into account, but they are important. Even within the same subfamily, the intraspecific variation may vary by several percent. Besides the lineage age of a species, it also makes a tremendous difference if the species is wingless or a flying one, if the distribution area is limited to some square meters or a wide area through several countries or a mainland versus island species.

Past subsistence practices in New Zealand revealed by ancient DNA

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Background: When Polynesian settlers reached the coast of New Zealand in the beginning of the 14th century they were met by a local fauna vastly different from what they had been accustomed to in Polynesia. Successful colonization and long-term survival on the islands of New Zealand must have required a swift adaptation to new hunting and fishing strategies suitable for the local fauna. Much of the information we have today about the subsistence practices of the first settlers of New Zealand is based on morphological analyses of bones excavated from midden deposits. Furthermore, morphological analysis is only applicable to a small fraction of such excavated bones, as fragmented and non-diagnostic bones constitute the vast majority of typical midden assemblages. **Results:** In order to study Maori subsistence practices, using a larger part of the bone assemblages, we analysed ancient DNA extracted from bulk bone samples across New Zealand. In total, over 6500 bone fragments collected from 26 archaeological and 14 natural sites were analysed. We found a highly diverse composition of species across all sites, with 195 different taxa, represented by a large group of bird species and smaller groups of fish species and marine mammals. Ordination analyses revealed a clear clustering of paleontological and archaeological sites, and, more importantly, a separation within archaeological sites along a North–South gradient based on fish species composition. **Significance:** As the first broad-scale survey of biodiversity based on bulk bone metabarcoding, this study highlights the advantages of the method. With the identification of previously unidentified species such as fin whale (*Balaenoptera physalus*) and longfin eel (*Anguilla dieffenbachii*) and the identification of well-known subsistence species such as fur seal (*Arctocephalus forsteri*) and Moa (Dinornithiformes), we demonstrate that a genetic approach reliably confirms previous results and provides new information to well-studied bone assemblages.

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Cracking down on counterfeits: creating a DNA barcode reference library of commercial herbal products traded in South Africa

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Background: Herbal products have been used for different purposes throughout human history, especially to treat numerous health ailments. Generally, it is believed that herbal products are affordable and safer to use compared to modern medications. The increase in the demand for herbal products places suppliers under immense pressure to deliver. Subsequently, commercial herbal products are often subjected to contamination or substitution of the main plant ingredient listed on the product label. This can result in reduced therapeutic potential and poses a serious health risk for consumers. Currently, there are no standard practices or systems available for the identification of species used in herbal products in South Africa, other than chemical analyses alone. As a result, the industry suffers from fraudulent and unethical practices. **Results:** A list consisting of 70 native plant species used in commercial herbal products traded in South Africa were compiled. All reference samples and look-alike species (1–5 individuals per species) were sequenced using the core barcoding regions *rbcLa* and *matK* to compile the DNA database. The database was then used to authenticate local products. **Significance:** This DNA barcode reference library, the first of its kind in South Africa, can provide pharmaceutical companies with a database against which they are able to compare their sourced raw materials and verify their authenticity.

Molecular identification of small and medium Neotropical non-volant and volant mammals in a biogeographic Chocó locality

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Background: Mammals have become one of the most threatened groups in the Chocó biodiversity hotspot due to habitat loss. A detailed species-level identification is the first step towards adequate conservation strategies in the region. Traditional morphological identification (TMI) requires high levels of taxonomic expertise and perfectly preserved material for positive identification; nonetheless, even in the presence of these two conditions, TMI is a challenging task for speciose clades with great levels of cryptic diversity (e.g., bats, rodents, opossums). To overcome the challenges associated with TMI, DNA barcoding has been developed as an inexpensive and effective tool to identify and describe the diversity of Earth. We herein present the results of a large-scale DNA barcoding project in one of the most species-rich biomes of the globe, the Colombian Chocó. **Results:** We constructed a reference library of 150 DNA barcodes for Neotropical small mammals (i.e., orders Rodentia, Didelphimorphia, Chiroptera) and demonstrate its use as an ideal complement to TMI for the identification of described and undescribed species. These barcodes helped resolve problems with morphological identification within multiple species complexes such as those of bat genera *Dermanura*, *Platyrrhinus*, and *Uroderma*. Our data produced important information about rare species, such as the first new record of *Ichthyomys tweedii* for Colombia. Moreover, elevated DNA divergences provide strong support (in addition to the presence of unusual morphological traits) for the recognition of undescribed diversity within Sigmodontine clades. DNA barcode identification showed that 23% of non-volant mammals (usually juveniles) where misidentified using TMI. **Significance:** This study is the first large-scale attempt to provide a reference barcode library for small mammals in Colombia. Additionally, useful information is presented herein for the recognition of cryptic Neotropical species. This effort is also crucial in generating species-specific conservation plans for the Biogeographic Chocó, one of the world's most important biodiversity hotspots.

Sisyranthus: a poorly known genus within Apocynaceae from southern Africa

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Background: The poorly known genus *Sisyranthus* is placed in the tribe Ceropegieae and subtribe Anisotominae (Apocynaceae: Asclepiadoideae). The genus was first described by Meyer in 1837 and last revised in Flora Capensis (1908), and since then, only one new species has been described. Currently it comprises 13 recognised species found in the grasslands of southern Africa, with one species restricted to Zimbabwe. In existing phylogenies, the subtribe Anisotominae has been under sampled, and broader sampling of southern African taxa is required in order to resolve relationships within and between *Sisyranthus* and its close allies. Furthermore, the existing key is difficult to use, thus leading to confusing identifications. Challenges in identification are related to diagnostic characters being hidden in the tube of the flowers, and they are further cryptic in both their habit and small size of their flowers. In this study, all species of *Sisyranthus* together with representatives within Anisotominae were barcoded, using the core barcoding regions *rbcLa* and *matK*, along with sequence data from two nuclear markers (ITS and ETS) and three plastid regions (*ndhF, trnL-F*, and *ycf1*). Morphological characters were reconstructed onto the phylogeny. **Results:** The resulting phylogeny indicates that *Sisyranthus* represents a well-supported monophyletic clade within the Anisotominae, with the genera *Anisotoma* and *Riocreuxia* moderately to strongly supported as sister clades. However, within *Sisyranthus* several taxa were reduced to polytomies due to a lack of informative sequence variation. **Significance:** The key produced is a crucial step to accurately identify species of *Sisyranthus* in the field. Furthermore, this study also provides the first step towards a much-needed revision of *Sisyranthus*.

Development of a rapid screening protocol to identify shark fins from endangered shark species

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Background: The collagen fibers from shark fins are the primary ingredient of the Asian luxurious delicacy shark fin soup, which is believed to have a number of health benefits. Trade of shark fins has driven worldwide overexploitation of sharks, threatening dozens of shark species. Effective April 2017, 12 shark species, including the oceanic whitetip (*Carcharhinus longimanus*), silky shark (*Carcharhinus falciformis*), the great white (*Carcharodon carcharias*), basking (*Cetorhinus maximus*), whale shark (*Rhincodon typus*), porbeagle (*Lamna nasus*), three species of hammerheads (*Sphyrna lewini*, *Sphyrna mokarran*, *Sphyrna zygaena*), and three species of thresher sharks (*Alopias pelagicus*, *Alopias superciliosus*, *Alopias vulpinus*) are listed in the Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). A rapid molecular method based on species-specific amplification and instant detection is needed to be developed for on-site identification. **Results:** In total, 175 samples from 58 species of shark in the form of fin, frozen tissues, and blood were collected locally and overseas. These samples include shark species commonly found in Asian sea areas and those closely related to the 12 endangered sharks. Species-specific PCR primers targeting the mitochondrial COI gene were designed for 12 endangered sharks. An efficient DNA extraction procedure for shark fin DNA extraction was developed for on-site detection. Loop-mediated isothermal amplification (LAMP) was

adopted for rapid identification. The result was revealed by color change of the reaction product. A lab-on-a-disc approach is being applied to streamline the process from DNA extraction to visualization of results. The developed protocol will allow rapid on-site identification of shark species, and for deciding if further in-depth investigation is needed. **Significance:** This is the first work on the rapid identification of endangered shark species and adapting the LAMP technique for shark species authentication.

DNA metabarcoding: application to common leopard diet

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Background: Metabarcoding is a rapid method of biodiversity assessment that combines two technologies: DNA taxonomy and high-throughput DNA sequencing. Short sequences of DNA are widely used to differentiate and assign taxonomies to specimens of animals, plants, and fungi and other microbes. **Results:** The common leopard diet was characterized from prey DNA present in faecal samples collected from Ayubia National Park, after amplification of a diagnostic fragment and sequencing of polymerase chain reaction (PCR) products, using next-generation (Illumina) sequencing. This provides diet information without any prior knowledge about the prey and is a cost-effective method as millions of read can be generated from a single sequencing run. This method has several advantages over classical microscopy, which requires substantial skill and time and is prone to misidentification in the case of closely related species. Of 111 putative faecal samples, 60 samples were identified as leopard. While three samples showed no prey item, eleven prey taxa were identified in the remaining 57 samples. Three prey items were identified in one sample, two prey items in seven samples, and a single prey item in 49 samples. Based on the frequency of occurrence of prey items in the 57 faecal samples, the domestic goat predominated the diet (64.9%), followed by dog (17.5%), and cow (12.3%). Domestic animals (goat, dog, cow, water buffalo; *Bubalus bubalis*, horse; *Equus caballus*, and sheep) occurred in 54 of 57 samples, corresponding to a frequency of occurrence of 0.95, and five samples contained two items of domestic prey.

Generic circumscription and relationships of southern African representatives of *Hypoxis* and allies (Hypoxidaceae, Asparagales)

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Background: The popularity of *Hypoxis* L. as a medicinal plant has resulted in unsustainable harvesting practices of rhizomes from the wild. This exploitation has accelerated the need for correct species names and circumscriptions. However, species delimitation in *Hypoxis* is problematic, and despite several attempts, the systematics of the genus remains largely unresolved. This is mainly due to the lack of distinct morphological boundaries separating species. Here, we explore the generic circumscriptions of *Hypoxis* and allied genera within Hypoxidaceae using five plastid DNA regions (*rbcLa*, *matK*, *trnL-F*, *ycf1*, and *trnS-G*). **Results:** Findings from our study indicate that *Hypoxis* is not monophyletic and is represented by at least three distinct lineages. Using the phylogeny produced from the study, *Hypoxis* samples sold at traditional medicinal markets in South Africa could be successfully identified. **Significance:** We proposed to transfer *Rhodohypoxis* Nel back into *Hypoxis* as well as the newly described genus *Sinocurculigo* from China to *Curculigo* Gaertn.

Time, money, and voucher saver protocol: non-destructive high-throughput DNA barcode analysis directly from the bulk tissue samples

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Background: Morphology-based identification of biomonitoring samples such as bulk benthos is labour-intensive and time consuming, and it rarely supports species-level resolution. Regular DNA barcoding protocols of individuals through traditional Sanger sequencing or environmental DNA (eDNA) metabarcoding through high-throughput sequencing (HTS) involve a step of partial or complete homogenization of the tested sample. This homogenization step leads to loss of either a part or the entire specimen. Here, we introduce an inexpensive protocol for environmental barcoding without any physical disturbance of the specimen. **Results:** We evaluated multiple non-destructive direct PCR approaches on five bulk benthos and five Malaise trap samples collected from the Wood Buffalo National Park, Canada. We were able to efficiently amplify both mitochondrial (e.g., COI barcodes) and nuclear markers from the free DNA in the preservative media. The efficiency of the approach increased by developing an optimization strategy. Amplicons generated from bulk samples were successfully sequenced in an Illumina MiSeq HTS platform and produced biodiversity results comparable to samples treated using a typical DNA extraction approach. **Significance:** The non-destructive protocol presented here will allow efficient analysis of contents of bulk aquatic (benthos) or terrestrial (Malaise) biodiversity samples either for analysis of whole biota or by specifically targeting assemblages such as pathogens, vectors, and rare or endangered organisms. Additionally, because the physical characteristics of specimens in bulk samples remain intact, it is possible to examine and verify each individual through morphological or additional genetic approaches. This is a significant advantage and critical for adoption of DNA metabarcoding in a wide range of socio-economic applications such as environmental assessment and monitoring. Given the cost and labour associated with DNA extraction approaches, by eliminating this step our method also provides cost saving for eDNA barcoding analysis.

DNA barcoding the planktonic rotifers from Mexico: a review

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Background: Rotifers are one of the most difficult groups to barcode. Because of this, there are only 9637 records of these animals in the Barcode of Life Data System (BOLD), most of them mined from GenBank and mostly representing species of the genus *Brachionus*. In Mexico, we have refined the barcoding methodologies for this phylum. Currently, Mexico is the country with the most diverse records in the world, mainly representing Monogonta, followed by New Zealand. **Results:** In total, we have recorded 618 rotifers from different groups, representing 143 species, from the small *Lecane bulla* complex to the big *Aspanchna brightwelli*. Most of them form complexes of species, even inside Mexico, where the tropics are completely different from the central highlands. Moreover, several new species described from Mexico in the past as *Brachionus josefinae*, *B. araceliae*, and *Keratella mexicana* have been confirmed as valid species through DNA barcoding. Some other new species of the *Brachionus plicatilis* complex are in the process of description, establishing new standards based on integrative taxonomy. Comparing our results with other parts of the world indicates that most rotifers are not cosmopolitans, and all varieties or subspecies described could be true species. For example, when compar-

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ing the rotifers from Mexico and New Zealand, despite many Linnean names being the same, just one single shared Barcode Index Number was observed. All specimens formed well-defined clusters in each country. **Significance:** DNA barcoding shows enormous potential for understanding species distributions and speciation in rotifers, but the difficulties in working with them seem to hinder the advance. With improved methodologies, it is possible to get sequence information from a single specimen. We hope this will help in future research on this group.

Butterfly diversity in Asia's megacities

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Background: Urban development is strongly associated with biodiversity declines and extirpations. For insects particularly, urbanization can result in the fragmentation and removal of vital foraging and nesting resources. Nonetheless, urban green spaces, such as gardens and parks, can attain remarkably high densities of pollinators and other declining species, suggesting urban green spaces could provide important habitats for insects. Urban parks can provide important refuges for wildlife as well as opportunities for people to interact with nature and enhance human psychological well-being. Many studies about urban biodiversity have been conducted in cities in temperate regions, but few studies exist for other regions, including rapidly urbanizing countries of Asia. **Results:** We sampled butterflies from urban parks in China, Malaysia, and Thailand. Standardised butterfly sampling was conducted across four different microhabitat types at each park: (*i*) groves, (*ii*) hedges, (*iii*) flowerbeds, and (*iv*) unmanaged. All sampled butterflies were identified based on wing morphology and DNA barcoding. We investigated the relationship between butterfly species richness and park variables (age, area, distance from the central business district). Preliminary analysis suggested that most of the butterflies are common and widely distributed species. **Significance:** This study will complement ongoing global research on urban ecology. The findings will highlight and promote techniques in urban park design and plant management that can improve habitat restoration and conservation of butterflies (and biodiversity in general), which are currently lacking for Asian cities.

Recording of *Gyrodactylus salaris* by analysis of environmental DNA in water samples from several rivers in Norway

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Background: *Gyrodactylus salaris* is a freshwater monogenean ectoparasite highly virulent towards Atlantic salmon and has caused major damage to Atlantic salmon river strains, with near extermination of the host a few years after infection. Current methods for detecting the parasite are expensive and time and work consuming. **Results:** To improve a monitoring program for detection of the parasite, we here report a method for recording the presence of *G. salaris* using real-time PCR analysis of environmental DNA (eDNA) isolated with water from rivers. Using a specific barcode sequence for *G. salaris*, samples were analyzed from infected rivers, previously infected rivers that have been treated to exterminate the parasite, and rivers where the parasite has never been recorded. This method shows high sensitivity, and the analysis detects *G. salaris* DNA at all studied locations in infected rivers, whereas none of the water samples from the non-infected river contained DNA from the parasite. As a control, eDNA from Atlantic salmon and brown trout was detected in water samples from all rivers. **Significance:** These results are promising in the development of a tool that can complement existing monitoring methods for detecting the presence of the parasite *G. salaris* in rivers by recording eDNA barcodes.

The complete picture: an update on the rapid biological inventory of a temperate nature reserve using DNA barcoding

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Background: In 2015 a rapid, barcode-assisted all taxon biodiversity inventory was completed at the rare Charitable Research Reserve in Cambridge, Canada. Two approaches were used – a 7-month sampling program and a 24 h bioblitz, each employing a variety of collecting techniques. Delegates from the 6th International Barcode of Life Conference took part in the bioblitz, expanding the species inventory through collection and identification of animals, plants, and fungi. During the single week of the conference, 3502 bioblitz specimens were collected, analyzed, and their data released in a published manuscript, demonstrating how swift a barcode-assisted inventory can be. **Results:** Mass sampling using six standardized collection methods was implemented at rare from April–October 2015. Overall, 5577 Barcode Index Numbers (BINs, a proxy for species) were determined from barcoding nearly 50 000 specimens. In total, 3332 BINs were released with the first publication, and the subsequent two months of collecting resulted in 2245 additional BINs. Insects dominated the inventory with 4554 BINs, mainly Diptera (49%) and Hymenoptera (23%). Adding the BINs that were assigned to species using the Barcode of Life Data System (BOLD) resulted in a species checklist of 3348 animals, plants, and fungi. Using public data on BOLD, a near complete BIN reference library was created to represent all animal species known from the reserve. **Significance:** Standardized methods of sampling are easy to implement and gather large and diverse quantities of specimens. Coupling this mass sampling with DNA barcoding can provide a rapid taxon inventory, even in the absence of taxonomic specialists. One season of this approach can result in an impressive local checklist—this study has now made rare one the best-inventoried reserves in North America. Furthermore, this approach complements traditional surveys and provides valuable occurrence data for difficult and small-bodied groups often disregarded.

Comparison of approaches for rapid barcode-assisted invertebrate surveys at Rouge National Urban Park

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Background: The ability to rapidly measure the health of an ecosystem is becoming increasingly important with respect to effects of global climate change and human activity on the environment. Invertebrate diversity assessments facilitated by genetic analysis show promise as a rapid and effective approach to measure baseline data and subsequently monitor changes in local communities over time. However, many methods of capturing this diversity prior to genetic analysis are available, which can vary greatly in the amount of time, effort, and cost required. In this study, we assess the effectiveness of three collecting strategies (a 24 h bioblitz, a week of standardized sampling, and 20 weeks of Malaise trapping) employed within the Rouge National Urban Park in the summer of 2013. **Results:** In total, 43 924 individual specimens were sequenced for the barcode region of COI. Of these records, 38 145 met minimum sequence quality criteria, representing 4422 putative species or Barcode Index Numbers (BINs). Despite temporal and spatial overlap between the three approaches, only 183 BINs were found using all three methods. One week of standardized sampling resulted in the highest capture abundance (21 443) and second-highest BIN richness (2091) but required the maximum collecting effort (612 min). Conversely, the maximum richness (2225) and similar capture abundance (18 118) was detected with just 60 min

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of effort using 20 weeks of Malaise trapping. In comparison, 610 min of collector effort was employed during the 24 h bioblitz but resulted in the lowest capture abundance (4363) and BIN richness detection (1215). **Significance:** Our results indicate that while each DNA barcode-based biotic survey approach captured unique diversity of the invertebrate community, Malaise trapping presents the most valuable method for invertebrate surveys with potential for long-term site monitoring.

A marine genetic baseline study at St. Eustatius, Caribbean Netherlands

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Background: Naturalis Biodiversity Center organized a marine expedition to St. Eustatius (June 2015) to set up a marine biodiversity baseline, which can be used for future studies on biotic changes after reef disturbances. A DNA barcoding workflow and environmental DNA (eDNA) metabarcoding was embedded in this survey. **Results:** The roving diver technique was applied at 35 dive sites (presence/absence records per dive with ~60 min observation time, including photography and collecting of voucher specimens for DNA subsampling). The taxonomic expertise of 20 participants (including citizen scientists) covered Scleractinia, Alcyonacea, Hydrozoa, Porifera, Mollusca, Pisces, macroalgae, associated fauna, interstitial fauna, and metagenomics. Algal communities were explored to identify biodiversity patterns. We identified 154 algae species from 424 collected specimens and used UPA, LSU, LSU-Y, COI, *tufA*, *rbcL*, and *matK* as barcoding markers. We collected 681 macrofauna specimens and identified 234 species by using COI, ND6, 28S, and *mutS* as barcoding markers. Metabarcoding of water filters and sediments was performed with different primer sets targeting COI. Accumulated results from filter and sediments sampling resulted in the preliminary identification of 157 genera, 128 families, 81 orders, and 28 classes. One-fifth of next-generation sequencing (NGS) reads could not be identified. The metabarcoding identifications show little overlap with those of collected and barcoded specimens. The diversity observed with eDNA monitoring is significantly lower than with visual observations. **Significance:** This barcode reference database of the biodiversity of St. Eustatius will be the groundwork for a DNA-based monitoring tool. Identifications by metabarcoding are supplementary to visual observations and therefore add biodiversity value to the baseline. It also indicates those species lacking from the reference library, which can be targeted for future sampling.

DNA barcodes unlocking the phenotypic plasticity in adult and larvae: a case study in Ceriantharia (Cnidaria, Anthozoa)

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Background: The subclass Ceriantharia (tube anemones; members of Anthozoa), known for the beauty of its polyps, suffers from confusing taxonomy. One of the main taxonomic problems within the group is the existence of larval forms that have been named and accepted as valid species. A possible approach to solve this problem is the use of DNA barcoding. **Results:** This study compared DNA barcoding, morphological, and developmental data of larval and adult stages of two morphologically defined species from related genera, *Arachnanthus* sp. and *Isarachnanthus nocturnus*, from the same region (São Sebastião, São Paulo, Brazil). As expected, morphological data showed the classical specific division of both genera. Developmental data also indicated a clear divergence in larval morphology and growth. However, molecular DNA barcoding and other DNA markers (16S, ITS1, and ITS2) showed a total absence of variation in sequences in all samples. These results indicate two alternative scenarios: (*i*) if they are truly different species, the drastic morphological differentiation must have happend a very short time after speciation; (*ii*) if they are the same species, there is considerable phenotypic plasticity in the studied species. We defend the last one, also based on the occurrence of different reproductive periods, resulting in different larval and adult morphology. These results become more important after molecular data of other species of *Arachnanthus* and *Isarachnanthus* were included, as results of Pacific Ocean specimens indicate that there may be no division between these two genera. **Significance:** This study has revealed that many concepts of the taxonomic delimitation of cerianthids may be mistaken, as such levels of phenotypic plasticity were not recognized in the past. In Ceriantharia, taxonomic problems are not restricted to species level, but higher taxonomic levels also appear to have inconsistencies.

Assessing the alpha diversity of Lepidoptera through DNA barcoding at the Mogale's Gate Biodiversity Centre, Hekpoort, South Africa

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Background: Inventorying the resident biodiversity of protected areas takes substantial resources and time, but DNA barcoding has proven a useful tool to reduce this investment, particularly in hyperdiverse taxa and biomes. A survey of the diversity of Lepidoptera (moths and butterflies) occurring in the ~4000 ha private conservation area, Mogale's Gate Biodiversity Centre (MGBC), was initiated in 2012 and has employed DNA barcoding to accelerate its completion. **Results:** Over 5000 specimens have been collected from various grassland and savanna sites within the reserve, primarily during the Forum Herbulot conference, in just five collecting nights, 13–17 February 2012. Individual participants morphologically identified their personal collections of the site. A subset of these specimens, including many undetermined taxa, were assigned taxonomy through DNA barcoding and then combined with individual lists for compilation of a master checklist. DNA barcode sequences were recovered from 4179 (98.8%) individuals, representing 47 families and 1003 putative species or Barcode Index Numbers (BINs). Nearly half (457) of the BINs were unique to the Barcode of Life Data System (BOLD), and just over one third (365) were assigned to a species once the barcodes were queried against BOLD. **Significance:** Our study provides a valuable evaluation of an approach for assessing the alpha diversity of a hyperdiverse taxon, which suffers from incomplete taxonomy. The ability to accurately assess the diversity of such taxa in protected areas is vitally important for proper conservation, land use planning, and management.

How food diversity influences microbiota diversity

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Comparative studies of the gut microbiota of traditional populations imply that the past human gut harbored a more diverse microbial community than that of the typical westerner. This reduced microbiota diversity in modernized human populations such as ours suggests that perturbations associated with modernization (including industrialized and processed food, antibiotics, sanitized water, and reduction in dietary diversity) likely eradicated certain members of the ancestral human gut microbiota. This reduction in intestinal microbiota has been associated with human diseases. However, the ecological role and potential functional contributions of these bacterial species that

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co-evolved with us remain to be explored. The genetic analysis of fecal material represents a non-invasive way to study multiple aspects of diet and has been widely adopted in ecological research. The advent of high-throughput sequencing has simplified the characterization of complex fecal DNA and now allows for simultaneous characterization of the different aspects of the ecology of a species. However, one of the remaining challenges is our inability to directly associate gut microbiota diversity with food diversity. By the simultaneous assessment of the species composition of diet and gut microbiome through DNA metabarcoding we want to understand how changes in food diversity affect intestinal microbial diversity. Both shotgun metagenomic sequencing and untargeted metabolomics are used to gain insight into the community functionality connected to food diversity.

Barcoding a corporate backyard: 3 years at ResMed Malaise trap in San Diego

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Background: The Global Malaise Program (GMP) is an international collaboration between the Centre for Biodiversity Genomics and 44 international contributors gathering insights into detailed temporal and spatial information on terrestrial arthropod communities across the globe. Since February 2014, ResMed, Inc., a leading San Diego medical sciences company, remains IBOLas first corporate partner, deploying their San Diego Barcode of Life (SDBOL) Malaise trap for over 3 years using GMP protocols. The ResMed trap collects specimens in a landscaped 2 ha open space sculpture garden on their 4 ha headquarters site in central urban/industrial San Diego. **Results:** To date, 103 weeks of consecutive sampling acquired 15 625 specimens (range, 4–483 individuals/week). Of these, 80.5% of specimens were successfully barcoded, with 1000 putative species in at least 19 orders, generating 182 unique Barcode Index Numbers (BINs) for Barcode of Life Data System (BOLD). Species accumulation curves were similar to less urban sites, suggesting 1976 species will potentially surface with continued sampling. Accumulation curves for BINs versus the number of analysed specimens suggest that slightly less than two thirds (~61%) of the expected arthropod diversity has been captured. Additionally, collections possessed a high proportion of BINs that were represented by singletons. Comparisons with other projects within the San Diego Barcode of Life initiative showed relatively low similarity indices (avg. 0.057), characteristic for a disturbed urban site, but possibly an artifact given the incompleteness of the overall sampling effort. **Significance:** ResMed's corporate programs substantially built a critical mass of barcode reference data with SDBOL, providing a foundation for further outreach and investment in a global biodiversity hotspot, including their first complete DNA barcoding of a globally important regional flora, the San Diego Plant Atlas. ResMed inspired a novel San Diego City Library initiative scaling Malaise and LifeScanner projects using library infrastructure across 36 sites.

DNA barcoding and systematics of the southern African endemic genus *Gasteria* (Xanthorrhoeaceae)

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Background: *Gasteria* Duval is a small succulent genus native to southern Africa. It predominantly occurs along the coastal regions of South Africa, encroaching into both Swaziland and Namibia. The taxonomy of the genus has been contentious due to the uncertainty in the number of species it includes as well as the limited number of diagnostic characteristics available to distinguish species within *Gasteria*. Currently, 25 species and 12 varieties are recognized. Based on the morphology of the pedicels, the genus is divided into two sections: *G.* section *longiflorae* Haw. (narrow elliptical flowers) and *G.* section *gasteria* Duval (globose flowers). In the current study, existing molecular sequence data sets were supplemented by including all currently recognised species of *Gasteria.* This was done in an attempt to improve resolution within the group. **Results:** Representatives of all 25 species and 12 varieties of *Gasteria* were collected and sequenced for the core barcoding regions (*matK* and *rbcLa*) and the additional markers (*psbA-trnH*, *trnL-F*, *ycf*1, ITS1). Our results shows that the genus *Gasteria* was strongly supported as monophyletic. However, relationships among species based on barcoding alone is less satisfactory. The inclusion of additional markers improved resolution within the genus and highlights the morphological sections *G.* section *longiflorae* and *G.* section *gasteria* as not monophyletic. **Significance:** This study contributes to the first comprehensive phylogenetic insight into the taxonomic relationship within the genus *Gasteria*.

DNA barcoding of Arctic Chironomidae (Diptera)

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Background: Chironomids are among the most abundant and species-rich insects in the Arctic, and species-specific habitat requirements for many species make them suitable for monitoring of Arctic environmental change. Since they are difficult to identify to species level based on morphology, this insect family is usually not utilized to its full potential in biodiversity assessments. DNA barcoding works well for species-level identification of all life stages in Chironomidae. Thus, a robust barcode library of Arctic taxa provides a splendid tool for future biological monitoring of the Arctic. However, taxonomical challenges exist, and recent work on the fauna of Svalbard has shown that thorough taxonomical review sometimes is necessary to assign the correct name to a barcode cluster and thus a link to previous knowledge about distribution, life history, habitat preferences, etc. **Results:** This study provides a metadata analysis of the currently available barcode data of Arctic Chironomidae in the Barcode of Life Data System (BOLD). More than 13 000 COI sequences (>600 bp) from 457 named species exist north of the southern tundra border, forming close to 1100 Barcode Index Numbers (BINs). Thus, a large gap between the number of identified species and the number of genetic clusters exists. Closer examination of selected groups shows that some species have a wider distribution than previously assumed. Other species, thought to be widely spread, have genetically divergent populations. **Significance:** It seems that the effects of climatic change will first be obvious in polar regions, but how will we monitor impacts on biodiversity if we only have fragmentary knowledge of animal diversity in these regions? For chironomids, a step forward is to create a rigorous baseline for future monitoring of biodiversity changes. A well-sampled barcode reference library for Arctic chironomids is a good start for common understanding of the taxonomy of this group and a necessary step to monitor Arctic biodiversity change.

DNA barcoding of plants in Thai Herbal Pharmacopoeias as a reference for quality control of plant origins and herbal products

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Background: The use of herbs as medicine and dietary supplements in Thailand has increased dramatically in the past few decades. Au-

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thentication of such herb materials is important for the assessment of safety and efficacy. The increasing demand for raw materials might lead to the intentional or unintentional substitution with other plant species in herbal drug regimens. There are several methods for plant identification including macroscopic, microscopic, and analytical chemistry. However, morphological characterization and chemical analysis are time-consuming procedures and require a level of expertise. These challenges reduce feasibility for application in the herbal medicine industry. The molecular characterization by using DNA markers is the ideal method for identification and authentication of herbal materials. In Thailand, there were 44 monographs of herbs in Volumes I–IV of Thai Herbal Pharmacopoeias (THP) produced by the Department of Medical Sciences, Ministry of Public Health. However, there was no information on molecular characteristics to such monographs as a reference standard for identification. **Results:** In this work, genomic DNA of 44 plants listed in Thai Herbal Pharmacopoeias were extracted and amplified for nucleotide sequences including *matK*, *rbcL*, *psbA-trnH* intergenic spacer, and also ITS. The DNA barcode section was appended as a supplement to the THP by our group, which was funded by The Department of Thai Traditional Medicine and Alternative Medicine, Ministry of Public Health. The information of DNA sequences of these sufficient loci provides the species-specific barcode for identification of the botanical origins of different plant species. Unknown plant species were subjected to a test using the DNA barcoding method and were successfully identified. **Significance:** This is the first study that produced a reference library of DNA barcodes for plants listed in THP. Regulatory agencies may propose DNA barcoding for manufacturers and merchants to ensure the identity of raw materials and processed herbal drugs.

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Genome-wide DNA barcoding: new concept of species identification tool using next-generation sequencing

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Background: DNA barcoding is successfully applied in many animal groups but still challenging in plants. To provide a breakthrough, especially for plant DNA barcoding, we propose a novel concept of the DNA barcoding framework based on genome-wide information detected by next-generation sequencing (NGS). Using the universal multiplexed intersimple sequence repeat (ISSR) primers, thousands of genome-wide regions can be routinely amplified from a wide variety of genomes. Then the library can be simply sequenced using NGS, and normally more than hundreds of genome-wide regions can be detected as comparable genomic information. This approach, called multiplexed ISSR genotyping by sequencing (MIG-seq), is effectively and reliably applicable for a wide variety of species, including plants, animals, and fungi, using the same protocol without any prior genetic information and protocol optimization. Therefore, hundreds of the detected sequences can be applicable to DNA barcodes. To demonstrate the applicability of this framework, we present an example of the genome-wide DNA barcoding for species of *Neolitsea* (Lauraceae) from Southeast Asia, which are almost impossible to distinguish based on standard cpDNA barcoding. **Results:** In total, 11 809 regions were sequenced for 69, 2, and 1 samples of *Neolitsea*, *Actinodaphne* (as a closely related genus), and *Machilus* (as an out-group), respectively, and used for neighbour-joining clustering. In all cases, duplicated DNA samples were identified as the same clade, and different species were distinguished from one another, resulting in comparable data with morphology- and ITS-based classification. **Significance:** Our new concept provides a quick (3 days), simple (two PCR steps and NGS run), and economical (~US$15 per sample) approach for DNA barcoding that is applicable to various organism groups. We expect that MIG-seq based genome-wide DNA barcoding will become the technique of choice for the "next-generation DNA barcoding".

Examining the effects of exine rupture on DNA extraction efficiency in pollen metabarcoding

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Background: DNA metabarcoding of pollen has the potential to overcome many of the complications of traditional morphological identifications and the dwindling number of researchers able to perform them. Despite this potential, pollen metabarcoding must overcome the problems inherent in plant barcoding as well as those specific to pollen. One aspect imperative to the success of pollen metabarcoding is development of a standardized method of DNA isolation that produces high-quality templates for the wide variety of sample sizes represented in environmental samples. Mechanical disruption of the pollen exine is thought to have a significant effect on the quantity and quality of extractions produced. The resistance of an exine to rupture is species specific and is influenced by several different morphological features of the pollen grain and must be taken into consideration when dealing with mixed-species samples. **Results:** This study serves to address (*i*) the technical complication in exine rupture prior to DNA extraction and (*ii*) the influence step one has on the quality and quantity of DNA extraction and the ratio of sequences produced to the number of pollen grains present in the initial sample. Fifteen anemophilous species of pollen varying in size, shape, and aperture number were subjected to bead beating with different bead sizes and duration. Following this, the markers ITS2, *rbcL*, and *matK* were sequenced using extractions at 0%, 33%, 67%, and 100% rupture in single-species and mixed-species samples. **Significance:** There has not yet been a single best recommendation for disturbance of the exine of mixed-pollen samples. Also, there are conflicting results of the extent to which this procedure improves the concentration and (or) quality of DNA extracts. The results will aid in taking species-specific stochasticity into account and in developing standard best practice procedures for DNA extraction of mixed-pollen samples.

The Austrian Barcode of Life: metamorphosis from the pilot phase into an initiative

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Starting in July 2014, a 3-year pilot phase was funded by the Federal Ministry of Science, Research and Economy, with the target of developing The Austrian Barcode of Life (ABOL) into a nation-wide initiative (www.abol.ac.at). The long-term aim was to provide DNA barcodes for all species of fungi, plants, and animals recorded from Austria. A large network was created of Austrian institutions and experts dealing with all aspects of biodiversity research in Austria, crosslinked with international initiatives and platforms. The funds for the pilot phase additionally allowed for generating data in four groups of organisms: (*i*) vertebrates, (*ii*) butterflies and moths, (*iii*) molluscs, and (*iv*) parasitic worms. In mid-2017 the transition from the pilot phase to the overall initiative took place. The necessity of using a large number of funding tracks created challenges in coordination and management. Taxonomic expertise was pooled in organism-specific clusters. An accepted cooperative project of Austrian universities is key for starting a national ABOL-initiative. Successful acquisition of different funds will be necessary to achieve the common goal. We present experiences from preparing the nation-wide initiative along with some results from the first 3-year phase of barcoding the Austrian biodiversity.

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Genome skimming for intraspecific phylogeography

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Background: DNA barcoding has had a significant impact on biodiversity research. Recently, it has been proposed to use genome skimming data as an extended barcode. Genome skimming corresponds to low-coverage shotgun sequencing of genomic DNA. Usually, for plants, one gigabase of genomic sequences can provide complete sequences of plastid genomes (chloroplastic and mitochondrial) and nuclear ribosomal regions. Here, we applied a genome skimming approach to tackle the intraspecific phylogeography of *Cedrus atlantica*, an endangered conifer distributed in Morocco and Algeria. This approach allows to assess both the paternal (via pollen and chloroplast DNA) and the maternal (via seeds and mitochondrial DNA) phylogeographies. **Results:** The whole geographic distribution of the species was sampled, with five samples per locality. A total of 183 genome skims were obtained for *C. atlantica*, plus three of the related species *C. libani*. Per sample, we obtained a mean number of 10 million 125-bp sequence reads. Based on this dataset, it was possible to assemble de novo whole chloroplast genomes. The observed level of variation was quite low, with about 50 mutations discovered over the whole genome. The mitochondrial genome and the nuclear ribosomal tandem repeats are currently under study. From the whole set of nuclear sequence reads, it was also possible to extract many microsatellites that might be useful for analysing the population structure. **Significance:** To our knowledge, this study represent the first large-scale genome skimming experiments at the intraspecific level on a non-model species. Such a strategy has many advantages, including the possibility to work on whole plastid genomes, on whole nuclear ribosomal tandem repeats, and on a random set of single-copy nuclear DNA. The same approach can be implemented on animals.

Altitudinal variation of some hemi-parasitic plants of the western region of Cameroon

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Hemi-parasitic plants commonly known under the family Loranthaceae causes economic loss, which varies with the host plant. In African countries, particularly in Cameroon, fewer studies have been done on these parasites, despite their cancerous impact on host plants. There have been recent studies on parasite-specific host inventories, but studies on altitudinal distributions, as well as different phenological stages, are still underway. The objective of this study was to assess species of Loranthaceae and their hosts, as well as to propose control strategies. To achieve this, activities have been carried out in nine localities. During the inventory, plant samples of Loranthaceae and hosts were collected and identified. In this study, four species of Loranthaceae have been identified: *Agelanthus brunneus* (Engl.) Balle & Hallé, *Globimetula braunii* (Engler) Van Tiegh., *Globimetula dinklagei* (Engler) polhill & Wiens, and *Phragmantera capitata* (Spreng) S. Balle. Results also show that *P. capitata* is ubiquitous, while *G. dinklagei* is confined to altitudes ranging from 400 to 1200 m. Up to 2200 m altitude, no parasites have been recorded despite the fact that the disseminator bird can survive lower temperatures. Loranthaceae hosts are comprised of 18 perennials plants belonging to 16 genera and 13 families. The far most parasitized trees are from families of Lauraceae, Moraceae, Podocarpaceae, and Bignoniaceae. The mean parasitic rate is 55.4%. *Phragmantera capitata* and *G. braunii* cause 60.8% and 21.13% of losses recorded in the locality, respectively. The duration of different phenological stages (germination, fixation, and foliation) experienced on *G. dinklagei* is limited to 21 days, and the global phenological tendency seems to be common to the four species of parasites. As a curative method of control, communities usually prune parasitized branches or cut down highly infested trees.

Land perturbation and impact on plant biodiversity in the buffer area of Mbam and Inoubou division in Cameroon

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Approximately 80% of the Mbam and Inoubou territories are covered with a buffer landscape. This transitional area is dispatched into the secondary degraded forest, the savannah zones, cocoa-based agroforest plantations, fallows, and field crops. Floristic surveys were carried out in all these different land-use systems to assess the impact of land perturbation or conservation on above-ground plant biodiversity. Besides various diversity studies, plant density was measured, and diameter at breast height was estimated for trees and shrubs, while dominant herb density was estimated. The results showed that the forest areas, which represent the historic biodiversity of the region, are more diversified and preserve the greatest number of species (76 species). This degraded forest is still important in seeds' dissemination process that enriches the savannah zones' diversity. Results reveal also that the savannah area is shifting and more and more colonized with forest plants. Forest can, therefore, be considered as refuge areas for savannah and cocoa-based agroforest plant species that may function as a starting point for possible regeneration of original biodiversity. Species richness is reduced progressively from the degraded forest (76 spp.) and cocoa-based agroforests (58 spp), to a savannah area (43 spp), to an old fallow field (36 spp.), and to the field crops (35 spp.), where only weeds and crops contribute essentially to plant biodiversity. Also, the number of species that are used as multi-useful plant species (construction, food, and medicines) decreased with increased land perturbation.

Can DNA barcodes help improve higher-level systematics? Simulations and the *Polyommatus* blue butterflies (Lepidoptera, Lycaenidae) provide an answer

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Background: DNA barcodes have proved to be advantageous in modern taxonomy for species-level identifications and the discovery of cryptic diversity. Higher-level taxonomic categories are also subject to new rearrangements by means of molecular evidence, including relocation of species in alternative genera, or even in higher categories. However, the use of single genetic markers is generally insufficient for inferring deeper phylogenetic relationships. In this work, we assess the potential that DNA barcodes may have in improving higher-level classifications. We investigate the phylogenetic history of the hyperdiverse *Polyommatina* (Lycaenidae, Polyommatinae) butterflies. Prior phylogenetic work throughout this subtribe, including 109 representatives with nine makers, allowed for a full taxonomic revision of the group at genus level. In an extension of this dataset, 1090 barcodes are gathered, comprising about 80% of the known specific taxa. **Results:** We evaluate the approach of combining high proportions of DNA barcodes on multilocus-based phylogenetic frameworks. Using large simulated datasets, we assess phylogenetic accuracy of using DNA barcodes only versus using datasets with increasing percentages of specimens with multiple markers. We show significant improvements for partial datasets including low multilocus percentages, which are enhanced when placeholders are selected on a prior taxonomic basis. When applying this approach to a large empirical survey throughout *Polyommatina*, several cases challenge current taxonomic hypotheses, but DNA barcodes allow discovering intriguing deep divergent

lineages and placing uncertain taxa into genera, all over a highly reliable phylogenetic scenario. **Significance:** This study contributes a new value for DNA barcoding, as a powerful tool for higher-level systematic improvements in speciose groups with uncertain taxonomy. Overall, we illustrate the construction from zero of a molecular phylogeny for a large group of animals and how to deal with the unexpected implications for systematics.

Tackling microbial cryptic species problems using large-scale RNA-seq data analysis

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Microbial eukaryotes are among the most notorious groups for the enormity of cryptic species problems. This is mainly due to the paucity of morphological characters that can be used for taxonomic delineations. The extent of cryptic species in amoeboid eukaryotes was realized when a handful of molecular studies, based on a single or small number of molecular markers, questioned the traditional taxonomy. These studies reported two major cases of discordance between morphology and molecules in members of Amoebozoa. In one case, the same morphospecies are genetically distinct, while in other cases, morphologically different species are genetically identical. Here, we investigate an example of the latter case. Previously, two morphospecies of the amoebozoan genus *Cochliopodium*, *C. minus* and *C. pentatrifurcatum*, were indistinguishable using traditional barcodes such as the small subunit rDNA (SSU) and cytochrome *c* oxidase I (COI) genes, despite being morphologically quite distinct. Given the divergent morphologies, we analyzed large RNA-seq data in these two morphospecies, with a more in-depth analysis of over 10 000 genes to delineate these two species. We used a custom-developed bioinformatics pipeline to match genes across species and calculate intrastrain and interspecies genetic distances. We found that 90.6% of homologous groups studied showed an interspecific distance lower than the traditionally defined barcoding gap for the genus (2% divergence), of which 84.0% varied by less than 1% between the two species. Our in-depth study on 1124 groups containing housekeeping genes showed even higher similarity between the species, with 98% of groups less than 2% diverged from each other. Our bioinformatics pipeline can effectively identify and exclude divergent paralogs that can impede barcode analysis in RNA-seq data. Based on these results, we conclude that *C. pentatrifurcatum* and *C. minus* are the same species, and they should be synonymized. Our study also identifies several markers that can be used for DNA barcoding in Amoebozoa.

The Centre for Biodiversity Genomics: state of the archives

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Background: The Centre for Biodiversity Genomics (CBG) at the University of Guelph is home to a globally unique natural history collection supporting innovative biodiversity research within Canada and internationally. Comprised of three interrelated resources, specimen archive, imaging laboratory, and DNA archive, the CBG stores, creates, and shares vital reference material for diagnosing and discovering species, determining their relationships, and tracking change over time. **Results:** The Specimen Archive currently holds nearly 2.9 million specimen vouchers, which are all tied to digital specimen records and exact storage locations within the archive. Specimen information is available on an internal collection management information system, and online on the Barcode of Life Data System (BOLD), where it is linked to barcode sequence data. The Specimen Archive is supplemented with over 650 000 high-resolution images of representative specimens from each species. To ensure broad taxon coverage of images, a pipeline has been established that prioritizes new species for imaging. Further, 93% of specimens in the Specimen Archive have high-quality extracts in the DNA Archive, which includes 2.4 million extracts stored in an ultracold freezer bank. The combined vouchers of the Specimen Archive and DNA Archive represent 69% of specimens on BOLD, and contain at least one representative for 77% of the Barcode Index Numbers (BINs) on BOLD. Notably, the archives contain the sole representatives for 65% of BINs on BOLD. **Significance:** These invaluable resources to the scientific community are well-utilized by partner institutions, with over 287 000 specimens and 196 000 DNA extracts loaned or donated to 263 institutions from 54 countries since 2008. The CBG also remains committed to making samples openly accessible: specimen vouchers and their associated DNA sequence information are available publicly on BOLD, and efforts thus far have released over 1 million specimens to the Global Biodiversity Information Facility via our national node, Canadensys.

Use of DNA barcodes for sustainable management of Madagascar precious wood

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Madagascar has more than 40 species of *Dalbergia* and more than 200 species of *Diospyros,* which constitute the precious woods (rosewood and ebony). They are objects of illicit exploitation and illegal trade. Since 2010, all species of these two genera have been introduced in CITES Appendix 2 for better protection. One reason that permits the illicit exploitation and illegal trade of these timbers is the lack of clear and precise scientific information on the existing species occurring in Madagascar. False declarations during the exploitation and exportation of timber are frequently used by economic operators. Currently, the Scientific Authority of CITES-Flore (University of Antananarivo) is aiming to (*i*) determine the exact number of species existing in Madagascar and (*ii*) identify simple, reliable, and inexpensive methods for the identification of each species. A project has been launched with the aims to (*i*) collect representative samples of all existing species in Madagascar and construct a reference collection comprising of a herbarium specimen, a piece of wood, and extracted total DNA; (*ii*) determine the exact number of existing species and provide a database on each species: ecological status, biology, genetics, distribution, exploitation, trade; and (*iii*) develop simple methods for species identification using physical, anatomical, and molecular (barcodes) characteristics. Laboratories have been set up at the University of Antananarivo, including a laboratory of molecular biology, that will work mainly on molecular barcodes of precious woods of Madagascar. This project is currently in the first phase of the collection of reference samples. At the end of this project, the obtained results will contribute mainly to the sustainable management of these precious woods in Madagascar.

Incipient biogeography-linked speciation in coastal southern Africa: a challenge to DNA barcoding

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Background: Intraspecific genetic structure along continuous coastlines with multiple distinct bioregions often mirrors biogeography. This suggests that the same barriers that maintain distinct species assemblages also play a role in the evolution of new (usually cryptic) biodiversity, yet some species display no genetic differentiation across multiple bioregions. Support for the paradigm that this discrepancy can be explained by higher dispersal potential is inconsistent. To determine whether some cryptic species have evolved so recently that DNA barcoding is not sufficiently informative to identify early-stage speciation, we generated genome-wide data for a widespread coastal fish that is genetically homogeneous across multiple

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temperature-defined southern African bioregions on the basis of COI sequence data. **Results:** Numerous loci were identified as being strongly correlated with water temperature after accounting for spatial population structure, and on the basis of which the species is divided into three spatially distinct regional population groups. Critically, the ranges of these groups are delimited by the same ecological boundaries that define distinct phylogenetic lineages in co-distributed coastal species. **Significance:** Our results suggest that in coastal regions that lack physical dispersal barriers and show steep gradients in water temperature, even populations exhibiting no divergence on the basis of DNA barcoding methods may already be on a trajectory towards evolutionary divergence.

DNA barcoding in curbing illegal wildlife trade, frauds, and trans-national criminals

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International wildlife trafficking not only threatens iconic species and erodes global biodiversity but also compromises local, national and global security. Studies have shown that exploitation by hunting for trade and pet collection is the second greatest driver, after habitat loss, for the declining populations of many endangered species. Several studies have demonstrated the applicability of the DNA marker technology in identifying species from various seizures such as confiscated meat samples, cooked and dried meats, dried shark fins, egg shells, animal hairs, bone, ivory, rhinoceros horns, turtle shell, feathers, and fish scales. Here, we intend to present case studies of identifying seizures by various enforcement agencies, including identifying fully tanned skin, scales, and even the canned food products often sold in shopping malls, grocery shops, and also in the duty-free stores at airport premises. This talk will highlight the importance of authenticated references, DNA sequence data availability, and the application of DNA forensics in identifying species from the samples that often have no morphological integrity.

Naturalization of *Artemia* in the Indian subcontinent: molecular approach to delineate the diversity

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Among the different live feeds, *Artemia* are extensively used as the starter diet in the Indian larviculture industry since the 1980s. Mainly imported strains are used due to the lack of nutritional quality and optimum nauplii size of indigenous parthenogenetic *Artemia*, even though autochthonous parthenogentic *Artemia* populations have been reported from Indian hypersaline habitats since the 1950s. To assess the present status of the *Artemia* populations and the possibility of invasion by the introduced *A. franciscana* in Indian Salinas, an extensive study was conducted using conventional and molecular approaches. *Artemia* samples were collected from North-West, South-West, and South-East regions. The internal transcribed spacer-1 (ITS1) for the Indian population was PCR amplified and sequenced. The ITS-1 sequences of the Indian *Artemia* populations exhibited 99% homology with the exotic *A. franciscana*. The mean pair-wise genetic distances between the Indian *Artemia* populations were negligible, indicating their genetic similarity. The absence of any significant genetic distance values between Indian *Artemia* populations and *A. franciscana* confirms that they are conspecific. Phylogenetic analysis grouped all the Indian *Artemia* populations with *A. franciscana* species. Principal Coordinate Analysis (PCoA) clearly grouped the Indian *Artemia* populations and the reference strain of *A. franciscana* into a single cluster. While the remaining new world species, viz. *A. persimilis*, *A. salina*, and *A. sinica*, were grouped into another clade, the old world *A. persimilis* remained isolated. Widespread import and use of alien *A. franciscana* as live feed in hatcheries must have paved the way for its massive invasion followed by the displacement of *A. parthenogenetica* from the Indian hypersaline habitats. Lack of regional endemism in populations of distant origins was evident, indicating that the invasive populations have naturalized and are in the process of evolution. This forms the first report of invasion by *A. franciscana* in hypersaline habitats on the Indian subcontinent.

Ever since Gondwana: the influence of changing climate, fragmenting forest, and spreading savanna on the biogeography of African reptiles

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Continental Africa has experienced major biome shifts throughout the Cenozoic, with a progression from humid climate and pan-African forest to a mesic/arid, savanna-dominant landscape. This shift has major implications for the biogeographic history of fauna, particularly species that are forest specialists. Dated phylogenies were constructed for the lizard family Chamaeleonidae and the viper genus *Bitis* using multiple mitochondrial and nuclear markers in Bayesian and likelihood frameworks. The phylogenies for both groups show strong signatures of allopatric diversification for forest endemics in connection with periods of major forest fragmentation in the Oligocene (ca. 45 Mya) and later in the Miocene (ca. 15 Mya). Ancestral character state reconstruction for habitat type (forest, savanna, heathland, grassland, desert) shows that some clades have diversified into novel habitats at time periods that correspond to the emergence of those habitats (e.g., savanna, heathland, grassland, desert). Examination of ecologically relevant traits for these reptiles (e.g., gripping strength and limb kinematics for chameleons) from diverse habitats (e.g., forest, heathland) suggest that ecological diversification is a driving factor during these transitions to novel habitats through ecological opportunity. Thus, when these two reptile groups are examined over their entire evolutionary history, it is clear that their biogeographic patterns were influenced by both allopatric and ecological diversification due to the dynamic nature of biome shifts in Africa throughout the Cenozoic.

Authentication of freshwater pearls using next-generation sequencing

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Background: While all shelled mollusks can produce pearls, not all of them are valued as gems. Saltwater pearls produced by oysters of the family Pteriidae are among the most valuable and oldest gems. Most freshwater pearls are from mollusks within the order Unionoida (families Unionidae and Margaritiferidae). The majority of freshwater-cultured pearls are produced by species of the genus *Hyriopsis* (or their hybrids) that are commonly farmed in Asia. North America has a diverse molluscan fauna (~300 species) capable of producing natural pearls. Some natural pearl-producing US freshwater mollusks are rare and endangered due to pollution and disturbed habitats, and thus natural pearls often have higher value compared to cultured ones. Pearls cultured in domesticated freshwater mussels and saltwater oysters represent a billion-dollar industry that is in need of a technique to verify the species and origin of the pearls. **Results:** We generated a small reference library of DNA barcodes for pearl-bearing freshwater mollusks consisting of 15 records from the tissues and shells of the species *Amblema plicata*, *Fusconaia ebena*, *Lasmigona holstonia*, *Plectomerus dombeyanus*, and *Megalonaias nervosa*. Pearls of various sources were either micro-drilled or crushed using liquid nitrogen and mortar and pestle. We

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combined previously published DNA extraction protocols for pearls with newly designed primers for PCR amplification and next-generation sequencing (NGS) to recover ~100-bp fragments from 5 out of the 14 pearls. Four of the pearls were identified as *M. nervosa* and one to the genus *Potamilus*. **Significance:** This study provided assignment of individual pearls to oyster species associated with the pearl industry. The substantial difference in value between natural and cultured freshwater pearls makes our work of high interest to the jewelry industry. Since some pearls are bleached to improve their appearance, then further investigation needs to be carried out to determine whether bleached pearls retain detectable DNA.

Barcoding–HRM analysis for authentication of the medicinal plant *Bacopa monnieri*

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Background: Medicinal plants are consumed as a dietary supplement at an expensive price. The authentication of plants has been a concern in recent years because of the substitutions of plant materials in dietary supplements. *Bacopa monnieri*, herbals for cognitive improvement, were used in Ayurvedic Materia Medica for centuries. In Thailand, three species within the genus *Bacopa* (Plantaginaceae) are recognized, viz. *B. monnieri* (L.) Wettst, *B. caroliniana* (Walter) B. L. Rob., and *B. floribunda* (R. Br.) Wettst. Morphological characteristic resemblance of species in this genus makes it difficult to identify. Therefore DNA barcoding, a technique using plant DNA of short regions, provides a powerful tool for solving this problem. High-Resolution Melting (HRM) analysis based on nucleotide differences was applied to discriminate *B. monnieri* from other *Bacopa* spp. **Results:** Five candidate barcodes, *matK*, *rbcL*, *psbA-trnH* spacer, *ycf1*, and ITS, of the three taxa were successfully amplified and sequenced with universal primer pairs. The nucleotide polymorphisms of the five regions were used to distinguish among the three taxa. HRM analysis of the *ycf1* gene was performed. The constructed melting curves for *B. monnieri* and other species have significantly different clusters. The assay was effectively applied to commercial herbal products. **Significance:** HRM with specific primers has been developed using DNA barcoding for discriminating the important medicinal species, *B. monnieri*, from other related species. Also, the authentication is beneficial for quality control of medicinal plants to ensure the safety of the consumers.

Identification of natural product leads (Andrographolide) based on phylogenetic approach in genus *Andrographis* Wall. ex Nees using DNA barcodes

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Background: Plants have evolved with a high diversity of chemical compounds for their defences and survival that leads to the evolution of various specialized metabolites and its natural derivatives over a period of evolution. Phylogenetic approaches and studies on biosynthetic pathways offer a predictive approach to a selection of plants towards lead discoveries in traditional drug development. However, this combinatorial approach has rarely been tested, and usage of the phylogenetic signal for either new or alternative sources are poorly established. **Results:** We produced a phylogenetic hypothesis for the medicinally important plant genus *Andrographis* (Acanthaceae) based on maximum likelihood and Bayesian analysis of nuclear (ITS2) and plastid (*rbcL*, *psbA-trnH*, *trnL-trnF*) DNA sequences of over 20 species, of which 90% are endemic to peninsular India. We have investigated a labdane type diterpenoid - Andrographolide from the leaves based on high performance thin layer chromatography (HPTLC) and found evidence for a significant phylogenetic signal within the genus. **Significance:** The presence of this diterpenoid is monophyletic to subgenera *Andrographis*, and also the presence of various compounds in the chromatogram indicate metabolite diversity within this taxon. This has implications for the use of phylogenies to interpret chemical diversity, to select candidate taxa for lead discovery such as Andrographolide, and to make recommendations for alternate sources in traditional medicine and highlights conservation priorities.

DNA barcodes reveal micromoth true diversity and overlooked invasions in Madagascar

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Background: Of over 4600 described species of Lepidoptera in Madagascar, we characterise only ~1550 as "micromoths". Here, we provide an update of our efforts to profile the poorly understood Malagasy micromoth diversity, using DNA barcoding of light-trapped moths from 2011 in both natural and anthropogenically disturbed habitats, and a primary forest Malaise trap survey in 2014. **Results:** We successfully barcoded 2823 micromoth specimens belonging to 1488 Barcode Index Numbers (BINs), prioritising richness (singleton clusters comprise 69%). Specimens were identified using both morphology and the Barcode of Life Data System (BOLD). We were able to assign 94% of BINs to family, 34% to genus, and 6.6% to species level. Of the 43 different micromoth families found, Batrachedridae, Bedelliidae, Blastobasidae, Bucculatricidae, and Meessiidae are novel to the island. **Significance:** This is the first attempt to compile a reference library of DNA barcodes of Madagascan microlepidoptera. This study contributes 98% of some 1523 micromoth BINs from Madagascar on BOLD (as of March 2017); of these, ~1248 BINs are novel for BOLD. It reveals the extent of human impact (among the ~8% broadly distributed BINs, 54% are recognised pests, biocontrol agents, or likely invasive species, yet ~55 species of these are not in Viette's 1991 checklist). It strongly emphasizes the major effort needed to comprehensively document the remaining diversity of Madagascan microlepidopterans and the extent of biological invasion in tropical countries.

Biomonitoring tropical lakes using next-generation sequencing: the fishes of Lake Bacalar, Mexico

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Background: Lake Bacalar, the largest freshwater body in Quintana Roo State in the Yucatan Peninsula, hosts a diverse fauna including some potentially endemic species. Landscape transformation, pollution, and possible invasion by the Amazon sailfin catfish *Pterygoplichthys pardalis* are the three greatest threats to its biodiversity. Our goal was to develop a next-generation sequencing (NGS) protocol for fish environmental DNA (eDNA) applicable for monitoring of *P. pardalis* and the ecosystem health. **Results:** We optimized sampling and transportation protocols for water and sediments. In total, 61 species were recovered from water samples:

38 fishes, 2 amphibians, 12 birds, 6 mammals, and 3 reptiles; all previously recorded in the Bacalar watershed, except for the three fish species. These results indicate that our baseline is still incomplete. The eDNA recovered from water showed higher diversity in comparison to sediments (55 vs. 20 species). Nevertheless, certain species, such as crocodile (*Crocodylus moreletii*), were only recovered from the sediment samples. Most of the vertebrate eDNA operational taxonomic units (OTUs) recovered by Ion Torrent PGM were represented by low-coverage reads. The newly available Ion S5 platform was tested on selected water samples and generated 24× more coverage than the PGM. The sailfish *P. pardalis* was not detected in any of the field samples; therefore, to ensure its successful detection in the future monitoring, we conducted mock eDNA experiments. **Significance:** These results will be used to convince federal and regional authorities to develop strategies employing DNA-based methods to monitor biodiversity in Lake Bacalar and to demonstrate the usefulness of the BOLD reference database, where Mexico has contributed many records for fishes, reflecting the commitment of taxonomists in the Mexican Barcode of Life (MEXBOL) network.

DNA barcoding of ants from the Galapagos Archipelago: searching endemic and introduced species

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Background: Until now 50 ant species have been recorded from the Galapagos Archipelago. Yet, for more than half of them it is still unclear if they are native to the Galapagos or if they are alien introductions. This uncertainty is due to, amongst others, the fact that the ant fauna of mainland South America is far less studied, which renders it difficult to unequivocally infer the status of species in the Galapagos. To improve our insight into the status of ant species, we explore the possibility to use molecular variation in a fragment of the mitochondrial COI gene ("DNA barcoding fragment") as an additional tool to distinguish between introduced and native species. **Results:** Preliminary results suggest that the presumed endemic species *Camponotus planus*, *Camponotus macilentus*, *Pheidole williamsi*, and the still undescribed taxa *Pheidole* hh01 and *Nylanderia* spp. are indeed probably native species. For the species *Camponotus conspicuous zonatus*, *Pheidole megacephala*, *Hypoponera opacior*, *Hypoponera opacicpes*, *Monomorium floricola*, *Cardiocondyla emery*, and *Strumigenys louisianae*, all known from the continent, no COI variability was observed, which might indicate eventual founder effects as expected for recently introduced species. For *Nylanderia steinheili*, *Odontomachus bauri*, *Cyphomyrmex rimosus*, and *Monomorium* sp. nr *pharaonis*, too few specimens could be examined to assess the status. **Significance:** Our results stress that future studies should include a sufficient number of distinct populations from the archipelago and similar or sister species from mainland Ecuador to increase confidence in the status of a species. If these conditions are met, our initial results showed that COI may serve as an indicative tool to distinguish native from introduced species, even if mainland relatives are unknown. Nevertheless, lack of variation within COI might also be caused by other factors than recent introduction, and this will be discussed and illustrated.

Detecting alien invasive species in a Dutch harbor using eDNA

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Background: Commercial harbors and marinas are not only a main port for cargo vessels and yachts but also for botanical and zoological stowaways. Organisms are transported undetected from one location to another, via the intake and release of ballast water and via fouling of ship hulls. The mostly disturbed habitats in harbors offer a first place of settlement, from where they could spread into other areas. In this way, harbors and marinas form a stepping stone in the spreading of alien invasive species (AIS). **Results:** In this study we compare environmental DNA (eDNA) methods with traditional methods for detecting marine animals in a Dutch harbor, with an emphasis on AIS. Data were obtained by collecting and filtering 92 water samples from the Sloehaven of Vlissingen in the Netherlands, from different locations and water depths. Species lists were compared with available lists from Rapid Assessment Surveys and SETL-plates. First results from eDNA demonstrate 44 species, of which 15 species were not identified to species level. Compared to traditional methods we found 11 extra species, mainly worms and copepods that are living in the substrate or as plankton in the water column. However, we were unable to detect some common species groups such as sponges, see squirts, and bryozoans. Samples showed low species richness and low evenness. **Significance:** This is one of the few studies using eDNA from the water column to detect AIS in a marine harbor, for the full range of animal species. It demonstrates the potential of detecting species and AIS via eDNA. Primer bias and sampling methods highly influence the outcome of eDNA detection methods. Current results are supplementary to traditional methods, and some expected general species were not detected. Therefore, future research is aiming to diminish primer bias and introduce replicate sampling and amplification strategies.

DNA barcoding versus morphological taxonomy for the identification of oribatid mite communities

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Background: At present, characterization of oribatid mite communities relies upon extraction and morphological identification, with molecular approaches still requiring development. The aim of this study was to compare the efficiency and reliability of traditional morphological approach and DNA barcoding to identify oribatid mite species in a mixed community. Samples were collected along moorland transects and mites extracted and identified morphologically. Clone libraries were then prepared for each sample for the COI region and sequenced. **Results:** Twenty one samples, containing a total of 2200 oribatid individuals representing 44 morphological taxa, were sorted, identified, and successively cloned as mixed communities. Barcodes were obtained from 854 clones and assigned to operational taxonomic units (OTUs). New barcodes were generated for those species without a reference barcode in public databases. The results clearly show the relevance of body size and abundance for DNA recoverability from a pool of species. **Significance:** This is the first attempt of using COI to identify oribatid species from a mixed community. This study highlights the usefulness of an integrated approach to reach the best oribatid mite species discrimination results, and reveals the advantages and the limits of both methods.

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Development of DNA barcodes for the identification of plants from Amazonian metalliferous rocky outcrops

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Background: Mining activity within the Carajas National Forest in the Brazilian Amazon region requires the meticulous management of natural resources, with special attention given to the metalliferous rocky outcrops (Canga). Currently, biodiversity assessment is costly and laborious. In addition, it is a long process with a high degree of uncertainty in attributing species assignment, due to the lack of knowledge of the local flora. There is an urgent need for an inclusive understanding of the flora and the application of DNA barcodes for a more rapid and effective biodiversity assessment. **Results:** This study constructs a reference DNA barcode library for the flora of Carajas with a focus on the Canga. To achieve this goal a reference flora of Carajas was produced, and in parallel every specimen was submitted to DNA barcoding with eight different markers. Over 3000 specimens were barcoded, producing more than 5500 markers, corresponding to 134 families and 355 distinct genera. In order to streamline the process, we developed an automated DNA barcoding procedure and analytical pipelines. We have also constructed a database that includes all georeferenced barcoded and historical specimens. For species of additional interest, chloroplast and nuclear genome and transcriptome sequencing and landscape genomics are being routinely carried out. **Significance:** This is the first comprehensive DNA barcoding effort for plants in the targeted hyperdiverse Amazon region. We have provided an inclusive database with a focus on the Canga of the National Forest of Carajas. Carajas is an extremely mineral-rich province, and enabling the responsible and sustainable exploration of these natural resources is the mandate of public and private stakeholders. This work will support sustainable actions enabling the description, monitoring, and conservation of the local biodiversity.

Evolution of the freshwater sea snake *Hydrophis semperi* Garman, 1881 in Taal Lake, the Philippines

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Background: *Hydrophis semperi* is one of only three species of sea snakes that have transitioned from a marine to a freshwater environment. It is found in Taal Lake, the Philippines, which has been isolated from the surrounding marine environment for 260 years. Linear morphometrics, geometric morphometrics, and molecular techniques are used to identify and trace the evolutionary history of *H. semperi* to resolve its taxonomic and ecological status. This will contribute to effective conservation and management programs. **Results:** Linear univariate measurements of four snake species were taken and analyzed through PCA. *Hydrophis semperi* had the smallest measurements, followed closely by *H. cyanocinctus*. Similarities were seen among *H. semperi*, *H. cyanocinctus*, and *Lapemis curtus* in shape and scale placement. However, only the first two share great similarity in head shape and eye, nostril, and mouth placement. CVA indicates *H. semperi* and *H. cyanocinctus* share similar head shape, with a Mahalanobis distance value of 7.4635. Twenty-seven cytochrome *c* oxidase subunit I (COI) sequences were generated from the four species; these were analyzed along with 15 sequences of three species from GenBank. Pairwise distance comparisons indicate that *H. semperi* and *H. cyanocinctus* are genetically similar, since an overlap is observed between the maximum distance within species (0.013471) and minimum distance between species when considered different species (0.001785). Such overlap is not observed when considering these individuals as the same species (0.06074334). Neighbour-joining and Maximum Parsimony trees of hydrophids based on 565 nucleotides of COI show that *H. semperi* and *H. cyanocinctus* group together monophyletically, with bootstrap supports of 85% and 100%, respectively. **Significance:** Taal Lake is an ideal natural laboratory to observe speciation. The question remains if resident species like *H. semperi* have fully speciated or are still speciating. This study aims to determine the evolutionary origin of *H. semperi*, resolve its taxonomic status, and create a reptilian model of marine-to-freshwater transition.

Barcoding of estuarine macrophytes and phylogenetic diversity of different estuaries along the South African coastline

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Until recently there has been a paucity of genetic research on estuarine macrophytes. Still in its infancy, DNA barcoding and phylogenetic diversity studies of these taxa have been identified as important particularly for conservation. DNA barcoding involves sequencing highly variable gene regions to aide in species identification, while phylogenetic diversity (PD) is a measure of biodiversity that incorporates the genetic differences between species. The aims of this research are (*i*) to barcode the dominant estuarine macrophytes and (*ii*) to determine the phylogenetic diversity of selected South African estuaries as a proxy for conservation prioritisation, where estuaries with the greatest phylogenetic diversity can be deemed more important in conserving the evolutionary information. Barcoding will involve sequencing selected gene regions for 65 important estuarine species. The chloroplast gene regions *rbcLa+matK* and *ycf*1 will be used as the plant barcodes. The gene region *ycf*1 is compared with the core barcodes because it has allowed for greater species circumscription. Preliminary analyses from GenBank-retrieved sequences indicate that most estuarine species have barcodes available for *rbcLa* (48 out of 65 species) and *matK* (38 out of 65 species), whereas there are no sequences available for *yfc*1. This highlights the urgency to barcode the species for which there are no gene sequences available that will ultimately be used to determine the macrophyte phylogenetic diversity of estuaries. This work will also contribute to the objectives of the International Barcode of Life (iBOL).

Barcoding barks, powders, and mixtures: the molecular analysis of medicinal plants traded at Tanzanian markets

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Background: Medicinal plants are commonly traded in Tanzania, where large parts of the population still rely on traditional medicine for their primary healthcare. Although several studies on medicinal plants have been performed in Tanzania, no study has so far attempted to identify all species sold on the local markets. Identifying these medicinal plants is challenging since vendors trade sterile leaves, barks, and roots that are often sold in powdered form to increase shelf life and to allow mixing on the spot. Local names often match multiple scientific species or have not been matched to any scientific species or genus. Often, researchers would accompany medicinal plant vendors into the field to collect medicinal

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plant vouchers for morphological identification, but this is time-consuming, season-dependent, and might lead to misrepresentation of the plants that are actually present at the local markets. In this study, we identify medicinal plants sold at the Dar-es-Salaam and Tanga markets using barcoding and metabarcoding. **Results:** Over 650 single-ingredient medicinal plants samples were purchased from the herbal markets in Dar-es-Salaam and Tanga. The samples were analysed using *matK*, *rbcL*, and nrITS barcoding. In addition, 83 mixtures, reportedly containing 2–40 plant species, were analysed using nrITS metabarcoding. Molecular methods proved to be successful for the identification of the majority of the powdered medicinal plants sold on the markets. **Significance:** This study is the first to identify the actual medicinal plant products traded at local markets in Tanzania. Furthermore, this study corroborates findings that DNA barcoding can be successfully applied for the identification of material that is unidentifiable based on morphology. Lastly, it shows that nrITS metabarcoding can be used to elucidate the composition of medicinal plant mixtures. These identifications can be used as a basis for quantitative market surveys and for identifying possible associated sustainability issues.

DNA barcoding as a tool for species identification in two phytophagous hoverfly genera (Insecta: Diptera: Syrphidae)

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The taxonomy of many hoverfly genera is still insufficiently explored, for example, the two speciose phytophagous genera *Merodon* Meigen, 1803 and *Eumerus* Meigen, 1822. The genus *Eumerus* is widely distributed in the Palaearctic, Afrotropical, Oriental, and Australian regions, with 256 described species. The genus *Merodon* comprises more than 160 species, distributed across the Palaearctic and Afrotropical regions. Diagnosis and identification of species of both genera is difficult because (*i*) existing identification keys are incomplete, (*ii*) a large number of the species are of obscure taxonomic status, and (*iii*) the nomenclature is often blurred. Recently, traditional morphology-based taxonomy has become more integrative and includes the use of molecular data, morphological features (including morphometrical data), ecological indices, and biogeographical parameters. The aim of our study is to determine the usefulness of DNA barcoding in species identification in *Eumerus* and *Merodon*. The identification accuracy was evaluated with phylogenetic reconstruction, viz. Maximum Parsimony, Maximum Likelihood, and Bayesian Inference. Our results show that in general DNA barcoding is an adequate tool for species identification and delimitation of both genera, and it will prove very important for improving their taxonomy, especially for complexes of morphologically closely related species. However, within the species complexes of *Merodon avidus*, *M. luteomaculatus*, and *M. melanocerus*, DNA barcoding did not differentiate among the morphospecies, and additional mitochondrial and nuclear markers have to be applied.

DNA barcoding poorly documented Afrotropical vertebrate faunas: prospects for conservation and one health

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Background: An increasing number of studies using DNA barcodes contribute to the discovery of Afrotropical vertebrate species. In cases that geographic sampling was adequate, these studies also provide increasingly detailed information on the distribution ranges of species for which this was till recently unavailable. **Challenges:** DNA barcode campaigns on poorly documented faunas require taxonomic expertise. Since taxonomic issues can only seldom be solved on a restricted geographical scale, the readiness to join forces to address taxonomic problems on a regional or continental scale is of prime importance to succeed. Experience has taught that the development of an adequate sampling campaign benefits from collaborations with local research teams. Moreover, such collaborations may also mitigate issues relating to the export of tissue samples (Nagoya Protocol on Access to Genetic Resources and Benefit Sharing). **Illustrations:** Several case studies demonstrate how collaborative efforts have yielded significant contributions to the inventory of small mammals (mainly rodents and shrews), fishes, and snakes from the Congo Basin. One example for Congo Basin fishes illustrates how the collaborative approach lowers the odds that independently generated reference DNA barcodes for specimens sampled from a poorly documented fauna may yield conflicting species identifications. **Prospects:** These studies compile reference libraries of DNA barcodes for a suite of smaller aquatic and terrestrial vertebrates that provide species-level identifications across tropical Africa. For some groups, the improved species distribution ranges contribute to a better assessment of the overall trends in extinction risk for groups of species (IUCN). Finally, because of the growing interest in emerging zoonotic diseases (transmitted between vertebrate animals and man with or without an arthropod intermediate), there is a growing interest in both the taxonomic and geographic diversity of small mammals, and the available tissue collections that can be screened for the presence of microbial pathogens.

Exploiting Alpine glaciers as biological archives: DNA metabarcoding of ice cores extracted from the largest and deepest southern Alps glacier, Adamello, Italy

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Background: Glaciers can be viewed as the most complete climate and environment archives, now severely threatened by climate change. These threats are particularly dramatic across the European Alps. The Adamello glacier is the largest, 16.4 km^2, and deepest, 270 m, Italian glacier. We aim at estimating biodiversity changes over the last centuries in relation to climate and human activities in the Adamello catchment area by introducing a new approach: DNA metabarcoding of ice cores. **Results:** Pilot drilling was conducted in March 2015: the resulting 5 m core has been analysed in terms of pollen spectrum, stable isotopes, and ions to determine the stratigraphy. The results showed that a stratigraphy is evident: this 5 m ice core is corresponding to ~5 years. DNA has been successfully extracted and amplified with specific barcodes: *trnL* cpDNA (primers d-h, about 150 bp) and a fragment of the mitochondrial COX1 (using three primer sets targeting the same region) have been used for investigating anemophilous plants and arthropod communities, respectively. Six libraries have been set up from three summer and three winter sections of the ice core. Plant metabarcoding not only confirms results obtained by morphological analysis but also demonstrates that ice cores provide a valuable source of eDNA, which allows identifications at species level. While most of the DNA is supposed to arise from pollen, in principle other material such as leaves might contribute to the total amount of DNA. Arthropod communities are mostly dominated by spiders, collembolans, and insects, the latter represented by dipteran species. **Significance:** The good preservation of eDNA in ice cores and the clear stratigraphy offers a unique opportunity to fully exploit the

promise of metabarcoding for assessing how biodiversity has changed through time in particularly sensitive areas of the planet in relation to the effects of climate change.

A DNA barcode reference library for the superorder Peracarida (Crustacea) from European coasts

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Background: The superorder Peracarida is a highly diverse crustacean taxon, comprising numerous prominent members in European coastal areas, communities, and ecosystems. Here, we present a core DNA barcode reference library for marine European superorder Peracarida, comprising specimens from the Black and Mediterranean Seas and from Northeast Atlantic coasts, ranging from the Iberian Peninsula to Scandinavia, including the Azores, Iceland, and the British Islands. **Results:** A total of 953 DNA barcodes were uploaded onto the Barcode of Life Data System (BOLD), of which 220 are new DNA barcodes. The dataset included specimens of the orders Amphipoda (67.2%), Cumacea (1.6%), Isopoda (25.3%), Mysida (4.1%), and Tanaidacea (1.8%). In total, 176 peracaridean morphospecies were assigned to 205 Barcode Index Numbers (BINs) in BOLD, with 163 (92.6%) represented by single BINs, comprising specimens collected from geographically distant populations, up to ~3500 km in the most extreme cases (e.g., *Idotea granulosa* from the Azores, Portugal, Iceland, Scotland, North Sea, and Norway). The remaining 13 morphospecies, belonging to Amphipoda and Isopoda, split between two to six BINs each, and had maximum intraspecific genetic distances between 3% and 25%. All multiple intraspecific BINs were allopatric, although the geographic distance between members of each BIN lineage ranged from 60 up to 3000 km. Major splits were detected between upper north and south regions of the Northeast Atlantic, between Atlantic and the Mediterranean Sea, or sometimes even within countries. The most striking case was revealed for the isopod *Janira maculosa*, which split into six BINs (maximum intraspecific distance 25.16%). **Significance:** The high percentage of morphospecies matching unique BINs (92.6%) shows the good reliability of this DNA barcode's reference library. However, the presence of deeply divergent intraspecific lineages suggests the presence of considerable overlooked taxonomic diversity. These findings indicate the need for a comprehensive revision and DNA barcode-based screening of the peracaridean fauna from the European coasts.

Challenges and opportunities in the globalisation of African traditional medicines: a South African perspective

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African traditional medicine is one of the oldest healing modalities supported by a wealth of ancient indigenous knowledge systems and modern scientific evidence. Despite a unique biodiversity and the extensive history of African traditional healing practices, very few commercial entities have been developed and globalised from the African flora. It is crucially important that a solid exploratory phase involving basic research should precede any commercialisation initiatives. Furthermore, developing phytomedicines based on traditional knowledge demands full compliance with local and international legislation. Several examples from the South African flora will be discussed to illustrate the challenging plant-to-product pipeline with emphasis on quality control issues and the need for complementary techniques to ensure quality, safety, and efficacy. Each of these species have an intricate history in the commercialisation process, and the importance of basic research and biosystematics studies will be highlighted as crucial steps in product development.

An integrated DNA barcode and ecological trait dataset for the Tuscan Archipelago butterflies: a resource to understand the evolution and extinction of island biodiversity

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Background: Islands disproportionately contribute to biodiversity, but their restricted habitats amplify the impacts of stochastic events and human-induced effects. Although island biogeography postulates the importance of integrating molecular data with species traits in order to understand the evolution of endemic taxa, the persistence of relicts and the extinction of populations, very few such studies exist for species-rich animal groups over entire archipelagos. **Results:** We assembled a comprehensive DNA barcode dataset for the 52 butterfly species reported from the Tuscan Archipelago in the western Mediterranean, including comparative material from neighbouring areas (Sardinia, Corsica, and Tuscany). We also compiled data on 10 species traits and on the disappearance of some of the butterfly species from the main islands of this archipelago in the last 115 years. We assessed (*i*) the phylogeographic structure of each species across the study area, as well as (*ii*) the overall phylogeographic pattern in the same region, and (*iii*) identified the traits associated with population diversification, uniqueness, or recent extinction from specific islands. There was a considerable degree of population diversification in many species, which confirms that the Tuscan Archipelago hosts highly diverse butterfly communities. Phylogenetic regressions showed that smaller-sized and more specialized species, with a preference for drier regions, display greater genetic structure and (or) uniqueness. Moreover, species adapted to colder and wetter areas and with shorter flight periods are more likely to become extinct. **Significance:** For the butterfly fauna of the Tuscan Archipelago, we analyzed fine-scale diversity patterns, their probable origins and the vulnerability of taxa to current and future environmental changes. The methodology used here represents a practical tool for evidence-based conservation prioritization, while the dataset provided serves as a resource for further research on island ecology and biodiversity.

DNA barcoding of the CBS collection: full speed ahead to bridge the gap in validated reference barcodes for fungal identification

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Background: Species identification tools form the basis of all biodiversity studies, and DNA-based approaches have now been incor-

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porated into biodiversity efforts to the expense of morphology-based approaches. Although barcoding has given rise to a rich study of bacterial biodiversity in every nook and cranny on earth, the lack of validated fungal barcode data is generally perceived as a setback for fungal research and applications. Being the largest fungal Biological Resource Centre in the world, the Westerdijk Institute initiated a barcoding project aimed at generating barcode data for all strains included in the CBS collection, and to create a major resource for fungal species identification. **Results:** Sequences of two loci, ITS and LSU, were generated for all (~80 000) fungal strains, originally assigned to ~17 000 species. Using the barcode sequences of ex-type and manually validated fungal strains, we were able to show that ITS and LSU can be used to identify up to 80% of fungal species with quality values of 84% and 78%, respectively. ITS was shown to outperform LSU in fungal species identification. However, LSU could be combined with ITS to provide improved resolution at species level. At higher taxonomic classifications, LSU was shown to have a better discriminatory capability than ITS. With quality values of 80%, LSU outperformed ITS in identifying fungi at order level. For class, family, and genus levels, the clustering quality values produced by both loci were quite low, indicating that there is a necessity for taxonomic revision at these levels. The barcodes of 4730 (51%) CBS yeast strains of 1351 (80%) accepted yeast species have been deposited to GenBank and made publicly available at the Westerdijk's website (http://www.westerdijkinstitute.nl). An additional barcode dataset of 4821 CBS filamentous fungal strains of 4089 accepted species is planned for imminent release.

Common ground: soil biodiversity and DNA barcoding

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Belowground ecosystems contain an estimated 25% of the earth's biodiversity, from bacteria to larger soil animals and plant roots. Despite this vast diversity, these habitats remained largely ignored compared to their aboveground counterparts. It is now well established that soil organisms provide essential services, including decay of organic matter, nutrient cycling, cleansing of water, and regulation of pests and pathogens. Our work globally has shown that soil invertebrates enhance litter decomposition by an average of 25% globally, and that threats to soils can impact soil biodiversity and ecosystem functions such as soil respiration. Unfortunately, complex relationships in soil and their benefits are often overlooked in management and policy decisions. The Global Soil Biodiversity Initiative (GSBI) as a scientific agenda was established in 2011 with the goal of advancing the knowledge of soil biodiversity science and implementing findings. To further advance our understanding of ecosystems, we need to be able to accurately identify and assess levels of biodiversity in belowground habitats. Ready access to global reference libraries of DNA barcodes (e.g., BOLD) will continue to simplify this process. However, an intensified effort is urgently required.

Alien invasive risk assessment of the marine aquarium trade in South Africa

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The marine aquarium trade, which is generally unregulated, is an ever-growing industry in South Africa. The movement of terrestrial animals and plants around the world has historically been responsible for most biological invasions and received considerable attention in policy and in scientific studies. However, the risk posed by the international trade in ornamental marine organisms in introducing alien invasive species has received far less attention. In this study, two possible vectors were investigated: (*i*) the import of marine fish, corals, invertebrates, and seaweeds through pet shop trade; and (*ii*) imported live rock that is known to carry pesky hitchhikers. The current poor standard of morphological taxonomy has resulted in the need to employ extra molecular taxonomy tools, specifically DNA barcoding. All hitchhikers sampled from the live rock and pesky organisms supplied by pet shops and marine aquarium owners were barcoded by amplification of the mitochondrial cytochrome *c* oxidase subunit 1 region. Population genetic parameters were explored to understand genetic diversity traits that could explain and possibly predict invasive potential. With this genetic parameter, extensive literature was also reviewed in order to conduct risk assessments on all species barcoded. Species that were determined to be of invasive risk were highlighted. These findings need to be explored further and should be considered when the authorities make regulation decisions. Overall, it is clear that stricter quarantine of imported species by the Department of Agriculture, Forestry and Fisheries is necessary, and that more accurate lists of species descriptions need to be supplied by international marine trading companies in order to clamp down on alien invasive species entering into South Africa.

The application of eDNA metabarcoding for marine biodiversity monitoring at the Cocos-Keeling Islands

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Background: Environmental DNA (eDNA) metabarcoding, a technique for retrieving multi-species DNA from environmental samples, can detect a diverse array of marine species from filtered seawater samples. With valuable applications in hazardous and logistically difficult to survey environments and in the detection of rare and cryptic species, this non-invasive technique shows great potential for use as part of a marine monitoring toolkit. **Results:** This study examined marine diversity across the Cocos-Keeling Islands, Australia—a remote coral reef atoll situated ~2950 km north-west of Perth, in the eastern Indian Ocean. This marine environment is unique in comprising both Indian and West Pacific species. Metabarcoding assays targeting the 16S rRNA, COI, and 18S rRNA regions of the mitochondrial and nuclear genomes, respectively, were applied to seawater samples collected from 56 study sites. Our assays have successfully detected a wide range of fish, arthropods, cnidarians, sponges, alveolates, and algae taxa present in marine water. Site composition varied over the 160 km^2 study region, notably in and outside of the Cocos-Keeling lagoon, illustrating a range of diverse marine communities and habitat preferences. **Significance:** Our research demonstrates the efficiency, cost-effectiveness, and power of using eDNA metabarcoding for comprehensive spatial biodiversity analyses, either alongside or in place of traditional surveying techniques. Building on rapid global and domestic developments in eDNA metabarcoding, it is expected that this genomic approach will soon be integrated into Australian marine resource management and consulting practices.

A new biosurveillance tool for a global problem: metabarcoding of environmental DNA to identify marine invasive species

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Background: Aquatic invasive species (AIS) continue to be redistributed globally and pose significant threats to biodiversity, ecosystem functioning, and economic growth. Molecular biosurveillance tools have the potential to alter the way we manage this global problem to better prevent or mitigate negative impacts. Current AIS detection and monitoring techniques are labour intensive and require a high

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level of morpho-taxonomic expertise. Here, we develop a powerful biosurveillance tool by combining metabarcoding with environmental DNA (eDNA) to detect and classify marine invasive species. This tool offers a high degree of taxonomic discrimination and a relatively fast turnaround for AIS identification. **Results:** Eight new metabarcoding markers were developed, two for each of four marine invertebrate phyla containing important invaders (tunicates, bryozoans, arthropods, and molluscs). Each marker was designed and tested in silico from publicly available DNA sequences to maximize taxonomic discriminatory power among both invasive and native congeners and to minimize non-specific amplification. Empirical testing on known samples determined that six of the eight markers together identified native and invasive species across all phyla, and they were henceforth considered successful for detecting invasive species of interest. The markers varied in terms of both amplification efficiency and success at species-level discrimination; however, at least one marker successfully identified known invasive species in each phylum from both environmental (water) and zooplankton DNA samples. **Significance:** Early detection and accurate identification of marine invasive species is paramount to the effectiveness of prevention, intervention, and eradication strategies. This novel tool will be applied in Canadian waters on the Pacific coast to showcase the power and promise of metabarcoding eDNA for the biosurveillance of marine invasive species in the highly interconnected and rapidly changing marine environment.

Developing a DNA barcoding pipeline for the identification and prevention of invasive plant propagules entering the Port of Savannah

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Background: Over 90% of global trade involves seaports that also serve as important points of entry for invasive plant species. The Port of Savannah, Georgia, is the fourth-largest and fastest-growing container terminal in the USA. The goal of this research is to increase the speed and reliability in the identification of propagules intercepted from shipping containers at seaports. Here, we (*i*) develop and evaluate the efficacy of a DNA barcoding pipeline to identify plant propagules based on their morphology and genetic identity; (*ii*) identify alternative sources of invasive propagules through the development of a local DNA barcode library; and (*iii*) evaluate the fitness of seeds recovered from shipping containers to determine potential invasiveness. **Results:** We collected 5582 seeds from the intake grills of 331 refrigerated shipping containers coming into the Port of Savannah between August 2015 and February 2017. Preliminary DNA barcoding and germination trials indicate a relatively high proportion (~70%) of the seeds collected from containers are from introduced and potentially invasive plant species (e.g., Cogongrass, *Imperata cylindrica*). Based on the port DNA barcoding library (~200 species), we found that ~30% were non-native and were not representative of the seeds collected from containers at the port. **Significance:** Our results have broad implications that will assist regulatory agencies in the prevention of invasive propagules entering ports. First, it is possible to identify seeds collected from containers based on their DNA barcodes; BLASTn analyses can confirm the identity of Federal Noxious Weeds not present at ports. Second, ports appear to be a critical yet cryptic point of entry for invasive species: the percentage of non-native flora at ports may be higher than averages for similarly disturbed areas. Finally, seeds intercepted at ports have the potential to become invasive; preliminary germination trials reveal that introduced seeds are viable.

Do functional and phylogenetic components of tropical tree diversity identify similar habitats for conservation priority on an oceanic island?

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Background: Islands are hotspots of both diversity and extinction: habitat modification, over exploitation, and invasions are some of the factors threatening these diverse natural habitats. The need for a rapid response to these factors necessitates a pragmatic approach towards the identification of conservation priority areas. DNA barcoding provides an effective tool for identifying habitats with high phylogenetic diversity and have often been used as a proxy of functional diversity when these data are unavailable. Here, we test whether measures of phylogenetic diversity are congruent with measures of functional diversity among tropical tree species on La Reunion island and how best these data can be used to identify potential conservation priority areas. **Results:** The congruence between functional and phylogenetic diversity is discussed, including how best these measures can be used in an integrative approach towards identifying areas for conservation priority. We further provide an assessment of the current boundaries of the National Park and whether these are effective at capturing important habitats. **Significance:** The study contributes towards a growing body of evidence documenting the relationship between functional and phylogenetic diversity and how these measures can be used in an integrative approach to provide recommendations for conservation planners.

Investigating the marine invertebrate fauna of the West African continental shelf with DNA barcodes

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Background: The University Museum of Bergen, Norway, has been cooperating with FAO's EAF-Nansen-Project (http://www.fao.org/in-action/eaf-nansen/en) since 2005 in exploring benthic invertebrate diversity on the continental shelf of the Canary Current (CCLME) and Gulf of Guinea Large Marine Ecosystems (GCLME). We have sorted and identified samples from ~700 sampling stations distributed from Morocco to Angola. With joint effort from African, European, and American partners, we have prepared about 2600 samples of predominantly Polychaeta, crustacean Malacostraca, Gastropoda, and Echinodermata for barcoding at CBG facilities. **Results:** To date, only 46% of the samples produced full sequences, but 385 of 618 submitted species are reported in the Barcode of Life Data System (BOLD) as complete. Of 2622 records, 1098 were automatically assigned to one of 480 Barcode Index Numbers (BINs). About 10% of the sequence records are singletons. A considerable proportion of the recovered sequences are not matching with data already hosted on BOLD and (or) GenBank. These figures partly reflect known endemic elements of the African Atlantic, but also discovering many undescribed species, particularly of less well-studied groups such as Polychaeta and Amphipoda. **Significance:** Barcoding and the BIN system of BOLD are particularly helpful in pinpointing similarities and differences among samples with reference to traditional taxonomic systems. Although the standard COI marker does not work universally it can, when it works, reveal misidentifications and taxonomic discordance among taxonomists in different scientific communities. If such discoveries are appropriately pursued through taxonomic revision it will contribute globally to better precision in accumulated species data. Barcode data have proven to be powerful tools in species discovery, indicating possible

needs for more integrative systematic studies aiming at better taxonomic resolution of evolutionary units of biodiversity.

Cycads tracked through DNA barcodes

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Background: South Africa is recognized as one of the hotspots for cycad diversity. However, it is presently at risk of losing 50% of its cycads within the next 2–10 years, a predicament described as "the South African cycad extinction crisis". This devastating loss of cycads, a tragedy largely unnoticed, is very similar to the rhino poaching crisis, with the largest threat been the illegal collection of plants from the wild to supply domestic and international trade. This has resulted in declines in cycad populations and even complete loss of subpopulations. Currently, of the 38 cycad species in South Africa (37 species of *Encephalartos* and one species of *Strangeria*), three are Extinct in the Wild, 12 Critically Endangered, four Endangered, seven Near Threatened, nine Vulnerable, and only three Least Concern species. Current legislation demands accurate identification for permit issue and successful prosecution, especially when traded products are degraded and morphological traits are not discernible. The project thus aims to develop standard operating procedures pertaining to collection, handling, and analysis of reference specimens for use in forensic investigations and development of a public free-for-use DNA barcode reference library of all species of *Encephalartos*. **Results:** A reference library, representing 64 of the 65 African species of *Encephalartos*, has been compiled. For each species, at least five duplicate samples from different geographical regions were sequenced using the core DNA barcodes (*rbcLa* and *matK*), as well as three additional regions *psbA-trnH*, nrITS, and NEEDLY. The DNA database has allowed for effective identification of cycad samples through comparing new crime scene samples to those already stored in the database. Several forensic case studies and challenges encountered will be discussed. **Significance:** The *Encephalartos* DNA barcode reference library developed from this study is the first step to combating cycad poaching and smuggling and to ensure successful prosecutions.

A review of over a decade of DNA barcoding in South Africa: a faunal perspective

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Background: For over a decade, DNA barcodes have been developed for species discrimination around the world. As of 2010, the vast majority of barcoding research was biased toward particular taxonomic groups and geographic regions, largely because researchers in developed countries were the ones with the resources and capacity to carry out such work. To rectify this, the International Barcode of Life Project was launched with the intent to extend the geographic and taxonomic coverage of the barcode reference library. **Results:** South Africa is committed to this mission in an attempt to catalogue all of its known biodiversity and, possibly, help identify new species. Approximately 48 000 South African faunal barcodes are housed in the Barcode of Life Data System (BOLD), which represent only 2.3% of all known South African animal species. Although insects are the best represented in absolute terms, with over 37 000 samples recorded, they are still grossly lacking, with just over 1% representation. Much like the global trend, there is a general taxonomic bias, with fish, birds, and mammals showing the greatest representation. Moreover, geographic bias is also present, with the Free State province particularly under-represented on BOLD, likely owing to limited human capacity. **Significance:** Although few studies have been published with respect to barcoding, the majority reveal that the cytochrome *c* oxidase 1 (COI) gene, used in isolation or in conjunction with other molecular markers, can greatly benefit South African biodiversity research. Several limitations of DNA barcoding are discussed and recommendations specific to South Africa provided.

DNA barcoding as a practical tool to assess the success of ecological restoration

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DNA barcoding has been used in a wide range of studies to understand and assess aspects that are related to ecology, evolution, conservation, and biogeography. In addition, it is useful in many different ecological applications such as species monitoring and ecological habitat restoration. Invertebrates are excellent biological indicators as they can be used to assess changes in species diversity or community assemblage in the context of restoration ecology. Understanding trends in species composition and assemblage of key invertebrate groups can provide important insight into the condition of, or changes in, the environment. In this study, DNA barcoding is used to assess the potential of Hemiptera as an indicator of restoration success for the Buffelsdraai Landfill Site Community Reforestation Project, Durban, South Africa. A total of 393 specimens were collected from sites reforested at distinct phases (2010, 2012, and 2015) and reference sites (forest and grassland). The Hemiptera species composition and assembly were assessed by analyzing multiple diversity indices, ordination, UPGMA cluster analysis, and phylogenetic analysis. A significant difference was found for Hemiptera species composition among the different reference sites as well as between 2015, 2012, and 2010 reforested sites. This study highlights the utility of DNA barcoding as a tool to monitor the success and progress of the reforestation and highlights the use of Hemiptera as a suitable biological indicator.

High-throughput terrestrial biodiversity assessments: PCR or PCR-free? DNA or RNA?

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There is growing interest in the use of high-throughput sequencing (HTS) of mixed terrestrial arthropod samples for biodiversity assessments. However, a consensus on the optimal approach used to examine the biodiversity present in mixed samples has not yet been reached. An approach to increase the proportion of taxonomically informative mitochondrial reads in HTS outputs, which has not yet been investigated in regards to terrestrial arthropod samples is "mito-metatranscriptomics". The objective of this study was to compare the utility of 16S rRNA metabarcoding (with PCR), mito-metagenomics (PCR-free DNA sequencing) and mito-metatranscriptomics (PCR-free RNA sequencing) approaches for detecting species in a mixed sample of terrestrial arthropods. The highest detection rate based on 16S rRNA was seen with the metabarcoding and nuclear rRNA-depleted mito-metatranscriptomics approaches. The highest detection rate based on cytochrome *c* oxidase I was seen with the mito-metagenomics approach, but mito-metatranscriptomics produced a larger proportion of relevant reads. Despite some increases in cost, mito-metatranscriptomics with nuclear rRNA depletion may offer considerable advantages over metabarcoding for terrestrial biodiversity assessments through reducing the number of spurious operational taxonomic units detected, while retaining high detection rates. Likewise, metatranscriptomics offers a natural enrichment of mitochondrial sequences, which may enable increased species detection rates compared to mito-metagenomics.

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Using DNA barcodes to monitor zooplankton community shifts following introduction of common carp

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We undertook a long-term field experiment to assess the effects of common carp (*Cyprinus carpio*) on zooplankton communities. In order to develop a more streamlined approach for the routine monitoring of zooplankton communities, we tested the use of DNA barcodes and corresponding metabarcoding approaches. These data were then contrasted with results from traditional morphological assessments. We obtained COI and 28S sequences from a range of taxa to build reference libraries. Possibly owing to the diverse taxonomic range for zooplankton (e.g., rotifers, crustaceans), the available primers for the COI region had a lower success rate than those available for 28S. Community samples were then analysed using the Illumina MiSeq platform. We found that all of the common species from each sample were adequately recovered using this approach. We conclude that next-generation sequencing approaches using COI and (or) 28S barcodes will provide a sensitive method to speed up and reduce costs involved in routine monitoring of zooplankton communities. This approach could potentially be applied to the detection of invasive species.

Phylogenetic analysis of Andean tree communities along an elevational gradient in Ecuador

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Background: Montane forests are known for their high diversity and endemism. Research on effects of environmental conditions on tree community structure in this habitat is limited because of resource constraints. The combination of traditional diversity metrics (Shannon's Index) and phylogenetic analyses can potentially reveal diversity patterns in montane forests generated by abiotic and (or) biotic factors associated with elevation. **Results:** In total, 595 tree samples (36 families, 53 genera, 88 species) were tagged, collected, and identified along a transect in the Andean Mountains at the Siempre Verde Reserve, Imbabura Province, Ecuador. Of these, 152 were DNA barcoded for the *rbcL* and *matK* gene regions. There was an inverse relationship between species richness and the number of stems of species that decreased and increased with elevation, respectively. At higher elevations there were fewer species, but there were more individuals of the same species. Results suggest significant clumping in the two highest elevation plots within the transect for phylogenetic diversity (PD), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD). These results correlate with the Shannon's diversity index, which indicates that species at higher elevations are more closely related. **Significance:** Previous research has linked phylogenetic clumping with habitat filtering. This study may provide a plausible explanation for why diversity peaks at mid-elevations where clouds begin to inundate the forest, causing vast differences in habitat above and below this elevation. As only four species span the entirety of the transect, abiotic stress could be the limiting factor in species distributions and a main contributor to the construction of plant community structure in non-random ways. To fully understand changes in biodiversity along elevational gradients, future studies should consider taxonomic diversity, functional diversity, and phylogenetic diversity.

Evaluation of the phylogenetic relationship between phytochemical presence and genetic diversity in tropical tree species

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Background: Only a small percentage of the world's flora has been adequately analyzed to determine its chemical composition. Treating phytochemical content as a trait measurement and combining this information with DNA barcode genetic sequences has the potential to lead to a better understanding of the occurrence and distribution of plants with medicinal phytochemicals. Using a combination of indigenous guidance and a prior knowledge of plant chemical composition, predictions of medicinal plant distribution, as well as the ecological and evolutionary mechanisms contributing to the assembly and diversity of tropical tree communities, can be made. The goal of this research was to evaluate the phylogenetic relationship between phytochemical presence and phylogenetic dispersion in tropical tree species of the Yasuni National Park in the Ecuadorian Amazon. Specifically, we constructed a tropical tree community phylogeny using DNA barcodes and tested for phylogenetic signal in the occurrence of phytochemicals. **Results:** Within Yasuni, 337 common tree species (56 families and 181 genera) were sequenced. Of these individuals, 248 species were successfully sequenced for the *rbcL* and (or) *matK* gene regions; 110 of these were classified as having the medicinal trait. Mean pairwise distance (MPD) and Fritz and Purvisa D statistic support a less than random distribution of the medicinal trait within the phylogeny, revealing potential ecological processes (direct competition, enemy-mediated density dependence) structuring the tree community of Yasuni. **Significance:** Preliminary findings indicate that a combination of ecological and evolutionary processes are controlling phytochemical distribution among tree species and that our use of DNA barcoding in a community phylogenetic context shows that traits such as phytochemicals are less than randomly distributed in lowland Amazonian tree communities. Increased species sampling along with the addition of other functional trait measurements is necessary to parse out which processes are playing large roles in tropical tree community dynamics.

Assessing the impact of reference library completion on the temporal and spatial patterns of wetland communities identified through DNA metabarcoding

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Background: Benthic macroinvertebrates are common bioindicators used in the assessment of aquatic ecosystems. It is well known that the number of specimens within samples, diversity of taxa, and range in size of specimens can make sample processing prohibitively slow, leading to the subsampling of individuals within samples—potentially altering the outcome of an assessment. The use of DNA metabarcoding allows for the identification of a range of taxa within samples with equal effort, which can be used to assess ecosystem state. Reference database completion can complicate assessments by potentially causing false positives or false negatives due to incomplete representation of taxa that occur within the sample. **Results:** We compared 78 wetland macroinvertebrate communities, identified morphologically to those same communities identified through DNA metabarcoding. To investigate how additions to the reference database impacted our data, the sequences obtained through next-generation sequencing (NGS) were compared against two reference databases of COI sequences downloaded from GenBank in September 2014 and April

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2016. We found an increase in accuracy of 11% between the two timepoints, which was purely from passive additions to the database. Spatial and temporal patterns in community composition did not differ significantly between the results obtained from the two databases. **Significance:** Although it did not affect the results of this study, we have highlighted the potential impact of an ever-increasing reference library on the environmental assessments made using genomic data. Mainly, if the reference library is incomplete at the time of assigning taxonomy to unknown sequences, the decisions made based on the data may change with the addition of new reference sequences to the database. Though the scale of this impact is unknown, further research into the effects of incomplete libraries on environmental decision making is needed—especially to establish when reference database coverage is adequate for spatio-temporal analysis of ecosystem state.

Biomonitoring for traditional herbal medicinal products using DNA metabarcoding and SMRT sequencing

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Background: The discovery of the anti-malarial drug Artemisinin raised the profile of Traditional Chinese Medicine (TCM), which was the source of this discovery that was awarded the 2015 Nobel Prize in Physiology or Medicine. This attracted worldwide attention and refocused attention on traditional herbal medicinal products (THMPs), especially the oversight of drug quality. Substandard and counterfeit THMPs are potential threats to public health. Recent marketplace studies using DNA barcoding have determined that the current quality control methods are not sufficient for ensuring the presence of authentic herbal ingredients and detection of contaminants and (or) adulterants. An efficient method for the authentication of herbal species in the multi-ingredient THMPs is greatly needed. **Results:** DNA metabarcoding and single-molecule, real-time (SMRT) sequencing was used to detect the multiple ingredients within THMP. SMRT sequencing was performed with the ITS2 and *psbA-trnH* amplicons using the circular-consensus sequencing (CCS) method. Several classical herbal prescriptions widely used in China were chosen to test the methodology. A biomonitoring standard operating procedure (SOP) was established using reference THMPs samples. The application of this SOP was tested on experimental mixtures and commercial THMPs products from the marketplace. The results suggest that it is repeatable and a reliable tool for detecting THMPs. The method was sensitive enough to detect all species, where ITS2/*psbA-trnH* amplicons could be sequenced in the THMPs, and the error in SMRT sequencing did not affect the ability to identify multiple medicinal species ingredients and several contaminants. **Significance:** This method represents the first trial of SMRT sequencing for the verification of multiple species mixtures in a THMP. Results suggest that this method has the potential of becoming a valuable quality control tool for monitoring the species composition of multi-ingredient THMPs.

High-throughput sequencing and bioinformatic analysis for multispecies identification in complex mixture products

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Background: The quality monitoring of traditional herbal medicinal products (THMP) is facing rigorous challenge in current traditional Chinese medicine (TCM) markets. The metabarcoding technology has been introduced into species identification of complex mixtures of THMP using second-generation sequencing (SGS). However, the rate of short-reads assembly and PCR errors relatively affects the identication accuracy of multispecies. Standard methods for THMP quality control is thus urgently needed. **Results:** This study supplied two strategies to solve the exogenous disturbance from high-throughput sequencing and PCR errors. First, direct covering and sequencing of PCR metabarcodes (ITS2 and *psbA-trnH*) was performed by single molecular real-time sequencing (SMRT) platform without assembly analysis. The circular-consensus sequenced (CCS) barcodes were extracted and clustered by perl scripts and CD-HIT software, and the clustered barcodes were aligned to the NCBI or DNA barcoding database for traditional medicine (http://www.tcmbarcode.cn) to identify the composition of THMP. Second, the whole genome of multispecies (metagenome) was sequenced using Illumina HiSeq platform. The short reads were mapped to the ITS2 and *psbA-trnH* database, respectively, and the mapping reads were assembled using genome assembly software, such as SOAPdenovo and VELVET. The assembled contigs were clustered and aligned to the NCBI or TCM barcode database to precisely identify the multispecies of THMP. These two methods effectively isolated and identified the composition from series THMP samples. **Significance:** This study introduced the sensitive and high-efficiency monitoring methods of biological composition in THMP using SGS or third-generation sequencing (TGS). And these methods will be potentially established to be the standard in the quality control of THMP circulation.

First reference library of DNA barcodes of earthworms of Kerala (a constituent of Western Ghats), biodiversity hotspot of India

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Background: The Indian state Kerala is a narrow strip of land spreading over an area of 38 863 km² along the southwest corner of the Indian subcontinent (between 8017′–12047′N and 74052′–77024′E). It is an important biodiversity region as 48% of its area falls under the Western Ghats (one of the eight hotspots of biodiversity of the world). Studies on earthworms of Kerala are sporadic and mostly faunistic in nature. **Results:** This study constructs a reference library of DNA barcodes of earthworms of Kerala from the Western Ghats of India. Around 350 specimens from 14 sites were DNA barcoded to delimit molecular operational taxonomic units (MOTUs). The MOTU number and composition were then used to estimate species richness. In total, barcodes have been generated for 45 species that have MOTUs with >10%–16% genetic distances. Most of them represented new DNA barcode records. *Amynthas corticis*, *Pontoscolex corethrurus*, *Metaphire houlleti*, *Plutellus* sp., *Drawida nilamburensis*, *Drawida ghatensis*, *Drawida travancorensis*, *Drawida papillifer*, *Drawida robusta*, *Drawida pellucida*, *Drawida papambikulamana*, *Drawida parva*, *Drawida sulcata*, *Drawida brunnea*, *Drawida willsi*, *Drawida kempi*, *Drawida chalakudiana*, *Moniligaster deshayesi*, *Megascolex insignis*, *Megascolex peermadensis*, *Megascolex ratus*, *Megascolex polytheca*, *Megascolex travancorensis*, *Megascolex trivandrunus*, *Megascolex konkanensis*, *Megascolex pumilio*, *Pithemera bicincta*, *Notoscolex minimus*, *Notoscolex ponmudianus*, *Glyphidrilus annandalei*, *Pontodrilus litoralis*, and *Dichogaster bolaui* were collected and barcoded. The barcode sequence itself was not found sufficient for robust phylogenetic analysis, though it allowed the detection of cryptic species, especially *Drawida* sp. DNA barcoding has helped to separate individuals with more complicated taxonomy of family, especially Moniligastridae and Megascolecidae. **Significance:** A reference library of DNA barcodes for earthworms of the Western Ghats of India was generated. The database may be helpful to identify earthworms even for scientists (non-taxonomists) working in the applied part of earthworm biology.

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Identification of ginseng in a murder case by the DNA barcoding approach

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Background: A new application for plant DNA barcoding involves forensic investigations. Examples of DNA analysis of botanical evidence include crime scene analysis and investigation of trade in illicit drugs. Plant evidence in soil plays an important role in burial site findings. In a case that occurred in the countryside, Jilin province, northeast China, a dead body was found near a stream. Soil was found adhering to the clothes of the body, which was apparently different from the soil of the stream site. **Results:** We extracted plant DNA from the soil using QIAGEN DNeasy plant mini kit. PCR amplification was performed for four regions: ITS2, *rbcL*, *matK*, and *psbA-trnH*. The nucleotide sequence alignment was based on the BLAST search. It was proven that the plant roots from the soil originated from *Panax ginseng*. Due to efforts of the police, the first burial site of the body was located at the suspect's ginseng planting field. **Significance:** The ubiquitous presence of plant species makes forensic botany useful for many criminal cases. DNA barcoding is a new technique that uses DNA sequences from a small fragment of the genome to identify species. This case illustrates that DNA barcode technology has played an important role in practical cases.

Cycad global diversity: explaining evolutionary history, historical biogeography, and predisposition to risk of extinction to inform conservation decisions

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Background: Cycads are a fascinating plant group sharing morphological features of ferns and angiosperms. Although they are the most threatened plant taxa, their predisposition to high risk of extinction remains to be elucidated. **Results:** I assembled the first complete phylogeny of cycads (339 taxa) combining DNA data and taxonomic information to investigate the evolutionary and biogeographic events responsible for the current diversity of cycads. I found that the present diversification is the results of multiple speciations and extinction shifts mediated by vicariance and dispersal events. I also fitted a cumulative link mixed effect model on biological, ecological, and evolutionary data of cycads. The diversity of threats and several variables linked to the biology and ecology of cycads correlate with extinction risk, and different variables seem to correlate with different IUCN status of cycads. In addition, species with higher evolutionary distinctiveness (ED) tend to be more at risk. Although their overall predictive power is generally <50%, geographic range and minimum diameter stood out as the best predictors for the vulnerable category, with a predictive power of 87% and 80%, respectively. I further showed that the loss of all vulnerable or endangered species does not depart significantly from random loss. In contrast, the loss of all top 50% ED, all threatened or all critically endangered species, would result in a greater loss of PD (phylogenetic diversity) than expected. Finally, I defined five hotspots of diversity, and depending on the diversity metric used, these hotspots are located in southern Africa, Australia, Indo-Pacific, and Mexico, and all are found within protected areas. **Significance:** This study used the first complete phylogeny of cycads to elucidate their historical biogeography and the pattern of extinction risk and demonstrated that the cycad tree of life would not survive the current extinction crisis.

The West African Center for DNA Barcoding of Fungi: progress, facilities, and challenges

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Background: Molecular methods have revolutionized studies in fungal taxonomy, ecology, and evolution. In addition, nucleotide-based systematics and phylogenies are also the major source for inference of macroevolutionary processes in fungi since fossils are rare and unevenly distributed between taxa. Despite the mega-diversity of tropical African fungi, they are still grossly under-represented in the international sequence databases (INSD); only 95 fungal ITS sequences from Benin, 300 from Nigeria, and 385 from Zambia compared to, e.g., 6445 from United Kingdom, 10 467 from Sweden, and 13 789 from Germany (UNITE database 24 April 2017). However, since a few years ago, numerous efforts have been made in West Africa to support international nucleotide databases with sequences from tropical African fungi, with the ultimate goal to facilitate the inclusion of African fungi in broad phylogenetic and evolutionary studies worldwide. **Results:** In this talk, we will present the state of molecular studies and phylogenetics undertaken on West African fungi, focusing on core symbiotic taxa such as Thelephorales, Russulales, Amanitaceae, Boletes, and Termitomyces. We will discuss facilities and challenges for the proposed West African Centre for Tropical Mycology that we attempt to establish at the University of Parakou, in collaboration with the University of Uppsala (Sweden), to promote a north–south transfer and south–south exchange of know-how on barcoding. Also we will discuss the significance of molecular techniques in applied mycology (identification of edible fungi and food security, of symbiotic fungi and forest regeneration, fungal ecology, functional diversity of fungi, etc). **Significance:** We will generate the first compilation of fungal DNA sequences in the West African region in an unified database to ease identification, phylogenetics, and evolution studies. We hereby evidence the need for deepening molecular studies on fungi, one important biodiversity component in Africa.

Water mites of the Great Lakes Watershed: exploring species boundaries with DNA barcodes

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Background: Although water mites (Trombidiformes: Prostigmata: Hydrachnidiae) are diverse and abundant in most freshwater habitats, the fauna is not well known outside Europe. At least half of the 2000 species known from North America are undescribed, and misidentifications are common due, in part, to the loose application of European names. In addition, many heteromorphic taxa are known only from a single sex and life stage. To address these issues, we employed DNA barcoding and the Barcode Index Number (BIN) system to delineate water mite species in the Great Lakes watershed. **Results:** Sequence analysis of 1600 specimens from 27 families, 50 genera, and 178 species revealed over 350 BINs. Almost 80% of the morphological species perfectly matched a single BIN, while only four species shared BINs and six were involved in BIN mixtures. However, BIN splits were detected in 30 taxa, several of which were supported by morphological differences. In some genera (e.g., *Limnesia*, *Lebertia*), the number of BINs surpassed the number of species currently known from North America. In addition, some species long presumed to have Holarctic distributions show clear divergence (>2% nearest-neighbour p-distance) from their European "conspecifics". **Significance:** This study provides the first large-scale DNA barcode reference library for water mites,

with coverage for nearly half of the genera and two thirds of the families known from the Great Lakes watershed. We detected far more diversity than expected, particularly in poorly studied genera. The barcode data also enabled the connection of dimorphic sexes and heteromorphic life history stages among mites, providing a framework for species description through integrative taxonomy in this group.

Exploring the diversity of Canadian mites with DNA barcodes

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Background: Although mites are diverse and abundant in most terrestrial and aquatic ecosystems, their species richness is poorly understood. Over 50 000 species have been described globally, but this total is thought to represent just 5%–10% of their true diversity. By providing an accurate, rapid method for species delineation, the Barcode Index Number (BIN) system can advance our understanding of their diversity. We used this method to assess mite diversity in Canada by DNA barcoding specimens from a large-scale sampling program with specimens from ~2000 terrestrial and freshwater sites across Canada. **Results:** We analyzed sequences of the barcode region of COI in 77 000 individual mites from all 13 Canadian provinces and territories. Among the 66 000 specimens that yielded a sequence >500 bp and with <1% ambiguous bases, we detected representatives of 6900 BINs belonging to all four mite orders (Ixodida, Mesostigmata, Sarcoptiformes, Trombidiformes) known from Canada. Species accumulation curves indicate that many more mite taxa are yet to be barcoded, a conclusion supported by the high frequency of singleton (36%) and doubleton (16%) BINs. Incidence-based BIN richness extrapolations indicate the Canadian fauna includes between 21 800 (Chao 2) and 27 300 (ICE) BINs. **Significance:** This study represents, by far, the largest DNA barcode analysis of mite diversity. The results suggest that there are presumably 10× more mite species in Canada than the 2855 species that are known, a total that is nearly 3× higher than the past estimate (10 000) for Canadian mite diversity. As Canada hosts ~1% of the world's species in most groups, the global mite fauna likely includes more than 2.5 million species. However, this inference will be further refined by sampling additional localities, particularly in the tropics.

MinION metagenomics: species identification and quantification of pollen collected from bees

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Background: Sequencing DNA from mixed samples of pollen to identify constituent plant species has important applications in palynology, allergy, and ecology. So far, protocols have been developed for amplicon sequencing (metabarcoding) of pollen, but metabarcoding is not robust to sample contamination. Also, pollen species quantification via PCR is subject to an unknown degree of error that can vary from sample to sample. Finally, an amplicon approach requires that samples be processed in a laboratory. We use the Oxford Nanopore MinION to sequence pollen samples collected from bees. MinIONs generate DNA reads of thousands to tens of thousands of base pairs long, but with high error rates (~10%–20%). To assign each read to a taxonomy, we generate a genome-skim reference database of 50 plant species and match kmers from each plant species reference "cloud" to each MinION read. The query sequences are long, and the reference sequences are short. **Results:** We show that this method allows reliable and semi-quantitative characterisation of the species composition of pollen collected from bees. **Significance:** It has been proposed that enrichment planting of food plants for bees will boost pollinator populations. However, bees are picky eaters, and there are dozens to hundreds of bees in any given agricultural landscape. MinION metagenomics of pollen could now make it possible to identify preferred plant species for multiple bee species without requiring a molecular laboratory except in the generation of the reference library.

Using Probabilistic Taxonomic Assignment (PROTAX) to census vertebrate wildlife from leech-derived iDNA

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Background: Nature reserves are the world's most important institution for conserving biodiversity, but we know little about whether these reserves are truly effective at protecting the species that live within them. There are two bottlenecks for censusing wildlife across large reserves: sampling and taxonomy. **Results:** We overcome the sampling bottleneck by using ranger-collected leeches from across a 678 km^2 nature reserve in China to census vertebrates: frogs, birds, and mammals. We overcome the taxonomy bottleneck by using amplicon metabarcoding of leech DNA extracts to detect residual vertebrate DNA in the leeches. However, taxonomic assignment has high uncertainty because DNA degradation forces us to amplify short genetic markers such as 16S rRNA, which have incomplete reference databases. We therefore report on the use of the new PROTAX pipeline for assigning taxonomies to DNA sequences, and we show PROTAX results in more reliable assignments, relative to other, better-known methods. Unlike other methods, PROTAX can take into account the uncertainty of taxonomic assignment caused by incomplete reference databases. **Significance:** Our long-term plan is to establish a protected-areas performance indicator by carrying out annual leech collections and mapping changes in species occupancies over time. Occupancy expansion and contraction are expected to correlate roughly to changes in population size. Invertebrate collectors of vertebrate DNA (known as iDNA) are widespread and can be mass collected and thus present a potentially very useful tool for censusing many species of conservation interest. Finally, the PROTAX approach could be of general benefit in DNA-based taxonomy.

Surveying non-arthropod diversity through DNA barcoding of arthropods

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Background: Besides their own DNA, organisms often carry genetic signals of other species they encounter and interact with. The sources of foreign DNA can be prey species, symbiotic organisms, or even environmental DNA (eDNA) acquired from the surrounding habitat. In many cases, the foreign DNA signals are too weak to detect using routine barcode analysis due to extremely low DNA yields and high levels of fragmentation. Rapid advances in next-generation sequencing (NGS) technology that permits simultaneous detection of multiple genetic targets with low DNA input have propelled metabarcoding research to the forefront of ecology and conservation biology by enabling dietary analysis, eDNA sensing, and other forms of non-invasive molecular tools for species diagnostics. Here, we explore the capacity of NGS-based barcode analyses to expose non-arthropod diversity by analyzing foreign DNA in arthropod samples. **Results:** By combining highly productive insect sampling techniques with the sensitivity of second- and third-generation sequencing, we analyzed over one thousand DNA extracts from both mixed and individual insect samples representing five major insect orders. Using primer cocktails targeting diverse phyla, we were able to simultaneously recover full-length barcodes for the target insect specimens and shorter sequences of foreign origin (the latter representing local vertebrate fauna, flora,

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and even pathogenic invertebrates). **Significance:** Our results suggest that large-scale arthropod surveys capture a much broader scope of genetic diversity than previously thought. In addition to being able to simultaneously detect the target arthropods and local non-arthropod flora and fauna, DNA extracts from arthropods of past surveys can be reanalyzed to gain a better understanding of the local diversity. Our method is fully compatible with high-throughput workflows for conventional DNA barcoding and thus enables the characterization of species interactions with high accuracy and unprecedented scale. Moreover, our data suggest caution in over-interpreting DNA barcoding results based on very short NGS reads.

Retrieval of genetic information from herbarium specimens

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Background: Herbaria are an unparalleled collection of biodiversity information representing the world's flora. However, this treasure has remained largely inaccessible to genetic studies, because of both generally limited success of DNA extraction and the challenges associated with PCR-amplifying highly degraded DNA. In today's next-generation sequencing (NGS) world, opportunities and prospects for historical DNA have changed dramatically, as most NGS methods are actually designed for using short fragmented DNA molecules as templates. **Results:** We used 25 herbarium specimens from 16 different Angiosperm families with ages up to 80 years old. A sufficient number of paired-end reads were generated, yielding successful chloroplast assemblies for 23 species and nuclear rDNAs for 24 species, respectively. This shows that genome skimming can be used to generate genomic information from herbarium specimens as old as 80 years and using as little as 500 pg of degraded starting DNA. We found no significant correlation between plastome coverage and nuclear genome size (*C* value) in our samples, but the range of *C* values included is limited. Finally, we conclude that routine plastome sequencing from herbarium specimens is feasible and cost-effective (compared with Sanger sequencing or plastome-enrichment approaches), and it can be performed with limited sample destruction. **Significance:** Our result is significant for the following reasons: (*i*) material otherwise not available, such as rare or extinct species, or costly to obtain is now within reach for comparative genomic analyses; (*ii*) availability of previously inaccessible genetic information from old type specimens that are crucial for resolving taxonomic uncertainties and for providing DNA barcodes for various applications (e.g., ecological studies, conservation, control of agricultural pests, and pathogens); and (*iii*) historical samples can provide insights into changes in genetic diversity over time.

Towards accurate species detection: calibrating metabarcoding methods based on multiplexing multiple markers

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Background: Metabarcoding approaches combine DNA barcoding of complex samples with high-throughput sequencing, often using one genetic marker and one generally universal primer pair. However, species-level identification depends heavily on the choice of marker and the performance of the primer pair, with a trade-off between amplification success and species-level resolution. We present a versatile metabarcoding protocol for biomonitoring that involves the use of multiple barcodes and multiple primer pairs per barcode in a single high-throughput run via sample multiplexing. A combination of three COI primer pairs and one 18S primer pair was selected for metabarcoding mock communities of zooplankton, giving three distinct COI fragments and one 18S fragment. **Results:** For the species detected by the COI marker, 75.0%–78.1% of species were detected by all three COI fragments, 16.7%–18.8% species by two COI fragments, and 3.1%–8.3% species by only one COI fragment. In total, the use of the COI marker resulted in the detection of 61.5%–82.8% species, while the use of the 18S marker resulted in the detection of 72.4%–75.0% of species. The percentage of species detected was significantly improved to 88.5%–93.1% by combining all four fragments of the two markers, with seven species being detected by a single COI and 13 species by the 18S fragment. Furthermore, the proportion of reads assigned to each species and the total number of species detected with the 18S fragment were similar when 18S was used alone versus combined with COI fragments in one Illumina run (i.e., mixing multiple amplicons from different markers and fragments prior to indexing). **Significance:** Overall, our metabarcoding approach utilizing multiple barcodes and multiple primer pairs per barcode improved the species detection rates of a single marker/primer pair by 13.5%–34.7%, making it an attractive, cost-effective method to biomonitor natural zooplankton communities.

Development of a reference standard library of chloroplast genome sequences, GenomeTrakrCP

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Background: An increasing number of people have turned to herbal dietary supplements for preventing diseases, staying healthy, and as an alternative to medicines after getting sick. In 2015, the total sales of herbal dietary supplements in the United States reached $6.92 billion, a 7.5% increase from the year before, and demand for botanicals have increased for 12 consecutive years. Many different chemical techniques have been used to monitor safety and authenticity of supplements, and more recently DNA-based tools have been included. While DNA barcoding has been found to be a powerful identification tool for many species, questions remain about its utility in plants, especially for processed products and closely related species. Therefore, developing methods targeting smaller diagnostic regions and reference libraries for rapid species identification of plants in foods and dietary supplements would be useful and complementary to chemical methods. In the past, the United States Food and Drug Administration (FDA) has been able to develop species-specific assays targeting plant species of interest by utilizing chloroplast genome sequences. **Results:** Presented here are the details for FDA's whole chloroplast genome sequencing effort and database, known as GenomeTrakrCP. Targeted species include plants found in foods and dietary supplements as well as known toxin producers, contaminants, or adulterants and closely related species to these. All data will be publically available through a bioproject in GenBank, e.g., PRJNA325670, derived from authenticated specimens and fully annotated. Currently, there are 40 complete chloroplast genomes in the database from authenticated specimens. **Significance:** These data can be used by the FDA and other government agencies, industry, and any other researcher as complete chloroplast genomes or to design species-specific assays to target plant species of interest.

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Phylogeny of coleoid cephalopods based on complete mitochondrial genomes and cryptic species identification with DNA barcoding in Octopodidae

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Background: Octopodidae is the largest group in Cephalopoda in terms of species. They are also one of the most confusing groups in international taxonomy and systematics, especially in the tropical waters of China where a large variety of groups remain poorly documented. Effective methods for the explanation of phylogenetic relationships and cryptic species delimitation are urgently needed. Character-based DNA barcoding and coalescent-based phylogenetic approaches provide a high-throughput solution to identify the cryptic or undiscovered species. **Results:** The complete mitochondrial genomes (mt genome) of five species, *Octopus cyanea*, *Callistoctopus luteus*, *Amphioctopus fangsiao*, *A. fangsiao* var. (with distinct different morphological characters from *A. fangsiao*), and *A. marginatus*, were sequenced, and the phylogenetic analysis across the class Cephalopoda based on the mt genome was conducted. The taxonomic results showed the monophyletic group of genera *Cistopus* and *Amphioctopus* and the sister group of *Cistopus* and *Octopus* (such as *O. vulgaris*). *Octopus minor* showed a very close relationship to *C. luteus*, but it exhibited a distant relationship to the other species in the genus *Octopus*. Therefore, we support the hypothesis that attributes *O.minor* to the genus *Callistoctopus*. Traditional phylogenetic diagnostic tools applied to the mitochondrial cytochrome *c* oxidase I (COI) gene and 16S rDNA and the mitochondrial NADH dehydrogenase subunit 5 gene (ND5) have proved to be more effective in exploring the high-level phylogenetic relationships in Coleoidea. Character-based methods and coalescent-based phylogenetic approaches with DNA barcoding indicated the existence of cryptic species in family Octopodidae along the coastal waters of China. **Significance:** With the rapid loss of marine biodiversity, this study provides a basis for the classification system of Octopodidae and first-hand information for molecular phylogenetics analysis of lower-level taxa, and molecular recognition of closely related species.

Understanding pollinator and pollination diversities using genome-skimming high-throughput sequencing methods

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Pollinators provide key ecological services to a variety of eco-systems. Characterizing the interactions between pollinators and flowering plants is the basis for understanding how natural and agricultural networks function. However, the following two fundamental questions remain difficult to answer: what pollinators are involved and what are they pollinating? DNA-based approaches, especially high-throughput sequencing (HTS) technologies, have demonstrated some promise in characterizing both pollinator and pollination diversities. In particular, PCR-free HTS coupled with expansion of classic reference barcode sequences to full-length mitochondrial or chloroplast genomes, i.e., genome skimming, have created potentials in identifying quantitative information for mixed taxa, in addition to providing species checklists. Compared with its cosmopolitan relative *Apis mellifera*, the Asian honey bee (*A. cerana cerana*) is more tolerant to low temperatures and adaptive to local flora. Although Asian honey bees are commonly believed to visit a much wider range of flowering plants, ecological records are scarce, and most evidence was based on visual observations. In this presentation, I will introduce some preliminary HTS applications in monitoring wild pollinating bees and progress in characterizing pollination diversity of the Asian honey bee.

Chloroplast genome structures and evolution analyses of two hemiparasitic species from genus *Taxillus*

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Background: Chloroplast genomic information is valuable in investigating plant evolution, barcode-based identification, and phylogenetic relationships among different species. The chloroplast genome of parasitic plants has many variations. The parasitic plants of Loranthaceae are hemiparasites that retain photosynthesis. In this paper, we report the complete chloroplast genome of two medicinal plants, *Taxillus chinensis* and *Taxillus sutchuenensis*, which are the first two complete chloroplast genome sequences of Loranthaceae. **Results:** The complete chloroplast genome of *T. chinensis*, which was divided into a large single-copy region (LSC, 70 355 bp) and a small single-copy region (SSC, 6082 bp) and separated by a pair of inverted repeats (IRs, 22 462 bp each), was a circular 121 361 bp-long molecule. The complete chloroplast genome of *T. sutchuenensis* was 122 562 bp long and retained a typical structure with LSC (70 630 bp), SSC (6102 bp), and two IRs (each 22 915 bp long). The GC contents of both species were 37.3% each. A total of 106 genes was identified from each genome. Each genome consisted of 66 protein-coding genes, 28 tRNAs, 8 rRNAs, and 4 pseudogenes, including *rpl*16, *rpl*2, and *ycf*15 (duplicate gene). All ndh genes, three ribosomal protein genes (*rpl*32, *rps*15, *rps*16), seven tRNA genes (*trnA-UGC*, *trnG-UCC*, *trnH-GUG*, *trnL-GAU*, *trnK-UUU*, *trnL-UAA*, trnV-UAC), four *ycf* genes (*ycf*1, *ycf*5, *ycf*9, *ycf*10), and the *infA* gene of the two species were lost. *Taxillus chinensis* and *T. sutchuenensis* were associated with *Schoepfia jasminodora* (Olacaceae), whereas three species of genus *Viscum* were grouped with *Osyris alba* (Santalaceae) based on the phylogenetic tree. The phylogenetic results strongly supported the theory that Loranthaceae and Viscoideae are separate evolutionary groups. **Significance:** Our results demonstrated the effect of parasitic lifestyle on the chloroplast structure and genome content of *T. chinensis* and *T. sutchuenensis*. The chloroplast genome also significantly contributes to the classification and evolution of species of *Taxillus*.

Identification of *Ranae oviductus* and its adulterants using COI sequences

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Background: *Oviductus ranae*, a valuable traditional Chinese medicine, is the dehydrated oviduct of *Rana temporaria chensinensis*. It is commonly used as an important tonic to replenish the kidney essence and relieve subhealthy fatigue in China, South Korea, Japan, and Southeast Asian countries. Because of its excellent curative effect and high price, there are many adulterants in the medicinal market, including the dehydrated oviduct of *Bufo bufo gargarizans*, *R. amurensis*, and *R. nigromaculata*. These adulterants bear similarity to *O. ranae* in appearance; however, they have a severe negative effect on clinical efficacy and medication safety. *Oviductus ranae* is easily intumescent and viscous in water, and it is difficult to extract DNA. In this study, COI sequences were used to identify *O. ranae* and its adulterants, which are difficult to identify effectively using traditional methods. **Results:** A total of 150 samples of the original species *O. ranae* and its adulterants were collected from different counties of Heilongjiang, Jilin, and Liaoning provinces in China. The results showed that the maximum

intraspecific divergence of *O. ranae* was less than the minimum interspecific divergence. A reference database was constructed based on the COI sequences of the original species *O. ranae* and its adulterants. Over 100 samples of *O. ranae* were collected from different medicinal markets, and the genomic DNA extraction procedures for these samples were improved. All the commercial samples were analysed using COI sequences. **Significance:** *Oviductus ranae* and its adulterants can be identified using COI sequences. We will use this reference database for practical inspection work, to strengthen the circulation and quality control of *O. ranae*, as well as to guarantee its clinical safety and better protect the legitimate rights and interests of consumers.

Using ITS2 barcodes to identify species of *Murraya*

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Background: Species of the genus *Murraya*, valuable plants for their characteristic aroma and medicinal value, have been used as astringents, analgesics, antidysenterics, or febrifuges in folk medicine in China and other Asian countries. According to Chinese Pharmacopoeia, *M. exotica* and *M. paniculata* are used as the original plants of Murrayae Folium et Cacumen, which is the main raw material in the drug Sanjiuweitai, with annual sales of ¥400 million. *Murraya koenigii*, commonly known as curry leaf or karipatta in Indian dialects, is widely used in Indian cookery for centuries and has been recorded in India pharmacopoeia owing to its versatile medicinal value. Furthermore, the essential oils of species of *Murraya* have also been utilized by the soap and cosmetic aromatherapy industry. However, there is some controversy about the classification and identification of species within *Murraya*. Here, we use ITS2 barcodes to resolve species identification within *Murraya*. **Results:** A total of 98 samples of eight species of *Murraya* were collected from different habitats in China, India, and Vietnam. The minimum interspecific genetic distances of *M. exotica*, *M. paniculata*, and *M. alata* were 0.014, 0.005, and 0.005, respectively, which were all less than the maximum intraspecific genetic distances of *M. paniculata* (0.018). Although the K2P genetic distance analysis cannot effectively distinguish them, there were four stable SNPs in ITS2 among these three species, which can be used to rapidly differentiate them. In addition, the UPGMA tree showed that different species can be differentiated according to their monophyly, and the identification efficiency of ITS2 barcode using BLAST was high. **Significance:** The ITS2 region could be used to rapidly and accurately distinguish Murrayae Folium et Cacumen from its closely related species, which provides an important contribution to its safe and effective clinical use.

Study on DNA barcoding of *Riptortus* (Hemiptera: Alydidae) in China

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Riptortus Stal is a small genus of Hemiptera: Heteroptera: Alydidae: Alydinae, consisting of 27 species. *Riptortus pedestris* and *Riptortus linearis* are two widely distributed species in China. In this study, a total of 144 samples of *R. pedestris* and *R. linearis* were sequenced. We used sequences of COI and Cyt *b* to analyse the genetic intra- and interspecific relationships between the two species. As a result, there was a significant interspecific genetic gap among the two species, and the distance between the species is much larger than that within species. The genetic analysis within each species shows that there was no significant subdivision between geographic populations of *R. linearis*, while the populations of *R. pedestris* are split into two clades and the distance between the two clades is not as significant as that of two different species. Evidence from morphological and geographical distribution suggested that the geographical barrier may not be the main cause of *R. pedestrisas* genetic differentiation.

Evaluation and optimization of DNA metabarcoding of aquatic invertebrates

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Background: Recurrent freshwater bio-assessments are important to achieve and subsequently maintain a good ecological status of aquatic ecosystems. The diversity and abundance of macroinvertebrates is a widely used bioindication tool in such assessments. However, morphological identification of these organisms is difficult, time consuming, and often requires sophisticated taxonomic expertise. DNA metabarcoding is an attractive, fast, and comprehensive alternative. However, the method currently lacks standardized protocols. As a subproject of GBOL II (German Barcode of Life II) we currently evaluate and standardize steps of the metabarcoding pipeline, focusing on laboratory protocols and bioinformatic analyses. These are further explained in this poster presentation. **Results:** Comparisons of three methods for sample tagging ("one-step", "two-step", TruSeq) revealed a high stability and potential of fusion primers for biomonitoring approaches, even in comparison to standard commercial ligation-based kits (TruSeq). The isolation of DNA directly from the samples' fixative (ethanol) was successful for insects in a mock community, while mollusks could not be detected in final sequencing results. The used protocol is currently applied to environmental samples to test its usability for biomonitoring and the susceptibility to inhibitors. Furthermore, results on samples without and with prior sorting from the substrate are presented using case studies from two stream ecosystems in Germany: the near-natural river Sieg and the highly altered and degraded river Emscher, now the target of one of the biggest restoration programs in Europe. **Significance:** The optimization and standardization of laboratory steps and protocols is key to the application of DNA-based approaches in routine biomonitoring programs. Thus, our results are of special importance not only for the standardized use of macroinvertebrate biomonitoring as part of the European Water Framework Directives (WFD) but for aquatic biomonitoring utilizing metabarcoding in general.

Agroforestry Parks around Park W: description, diversity of woody species, and preferences of local populations

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Protected area biodiversity conservation seems to be impossible without surrounding local population implication. The current study aimed at describing surrounding reserve agroforestry systems and highlighted the woody species diversity and local population preference. Three Communes (Banikoara, Karimama, and Kandi) and 135 farmers were interviewed. Two agroforestry practices were identified, namely forestry farming and silvopasture in association to crop yielding. Results showed that 37 plant species belonging to 15 families were conserved in agroforestry systems and used by the local population. In agroforestry systems, the most observed indigenous species were *Vitellaria paradoxa* and *Parkia biglobosa* associated with *Zea mays*, *Vigna unguiculata*, *Penicetum glaucum*, *Orysa* sp., *Arachis hypogaea*, *Glycine max*, *Gossypium* sp., *Sesanum indicum*, and *Sorghum bicolor*. Preferred species were *Vitellaria paradoxa*, *Khaya senegalensis*, and *Pterocarpus erinaceus* due to their importance. In total, 78% of interviewees declared to remove all indigenous species sapling with the aim of reducing shade on the crop and increasing their production. The main causes of reserve degradation were agriculture, livestock breeding, and logging. Interviewees suggested 32 species to re-forest agroforestry systems. The interviewees are aware of the state of degradation of the agroforestry systems.

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Global Genome Initiative: targeting taxonomic DNA barcode gaps in GenBank

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Background: The Global Genome Initiative (GGI) is a Smithsonian Institution program to collect, share, and study genomic samples of non-human species along the major branches of the Tree of Life. Specific goals include preserving genetic material of at least one species of half of the 160 000 – 200 000 estimated genera and sequencing 8000 DNA barcodes for taxa for which there is no genetic data available ("dark" taxa). GGI's barcoding strategy is guided by a data-mining approach in which GenBank is periodically queried to detect taxonomic groups that do not have sequences flagged as barcodes, thus allowing GGI to focus all sequencing efforts on lineages that are not represented in this repository. **Results:** To date, GGI has generated 2000 barcode sequences from four loci for more than 500 plant genera that are not currently represented in GenBank. Ongoing work preserving and sampling legacy collections of large plant diversity projects at the Smithsonian will yield at least 2500 additional barcodes for more than 650 dark plant genera during 2017. **Significance:** GGI's approach to barcoding is the first of its kind at the Smithsonian and will result in a substantial increase in the amount of barcode data available in GenBank for rare taxa from places like Tibet and the Guiana Shield.

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