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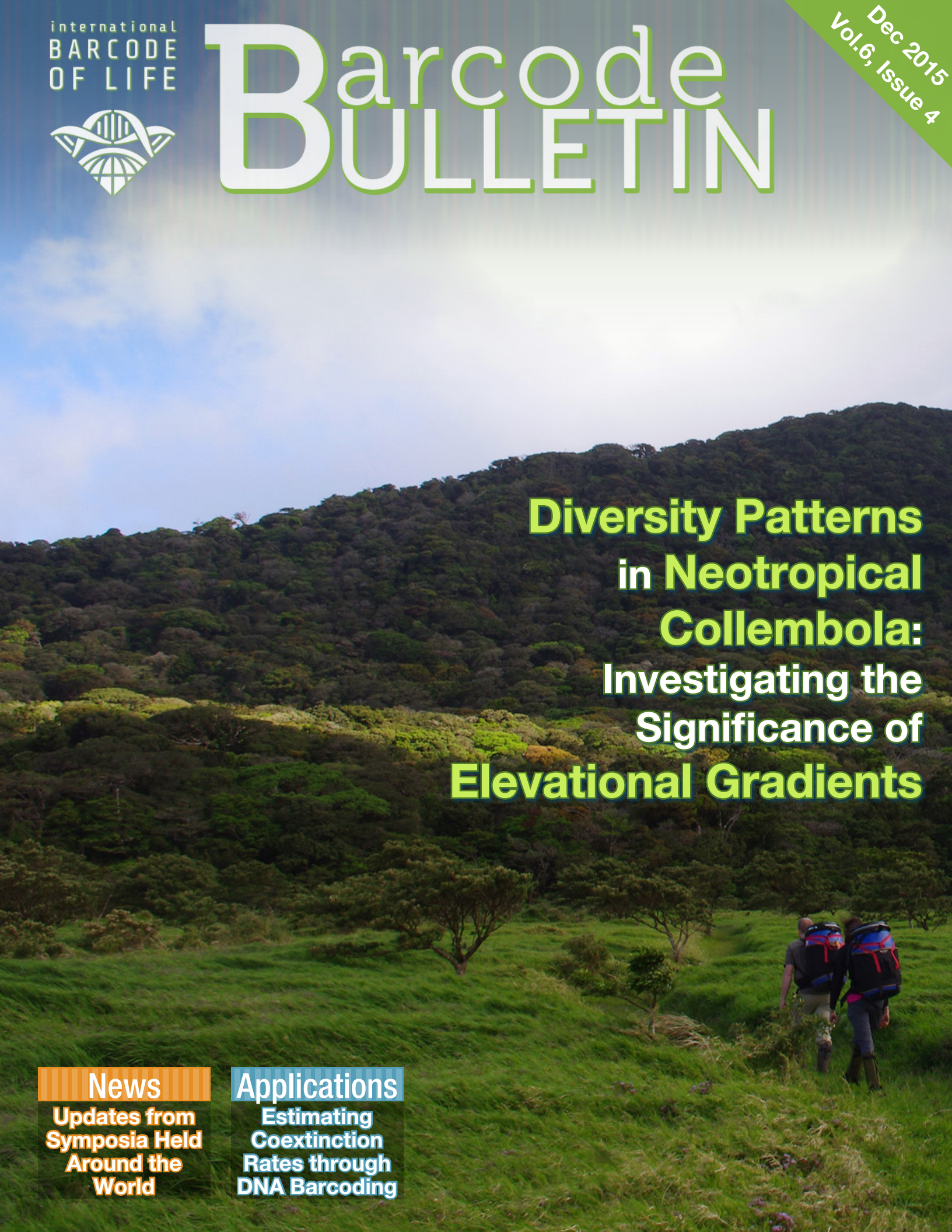

Diversity Patterns in Neotropical Collembola: Investigating the Significance of Elevational Gradients

News
Updates from Symposia Held Around the World

Applications
Estimating Coextinction Rates through DNA Barcoding

Welcome to our December 2015 issue.

Another eventful year has passed with the 6th International Barcode of Life conference as a fantastic highlight. 600 researchers from 50 nations, over 200 talks, more than 100 posters - far more than our little newsletter can ever convey even in a year with 4 jam-packed issues. Nevertheless, we are looking back at another successful year, and we will try to keep the momentum going that the conference started.

This issue contains more prize winners from the conference and a lot of good news with respect to funding and national initiatives.

We wish you a happy holiday season and a healthy and prosperous New Year.

Dirk Steinke
Editor-in-chief

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News Briefs

The Slovak National Museum-Natural History Museum obtained financial support of 1.7 M € from the EU European Regional Development Fund for building a DNA lab and other infrastructure to barcode the flora and fauna of Slovakia in 2016 – 2023. With the added capacity, the museum plans to barcode 1000 species in the coming years.

The German Barcode of Life Network (GBOL) was awarded a further 6.3 M € by the German Federal Ministry of Education and Research to extend the German barcode reference library to contain all common and frequent species, as well as important agricultural pests, invasive, health-relevant, Red List, FFH (Flora Fauna Habitat Directive), indicator and specific application-relevant species, and to develop DNA barcoding applications.

Through support from CONICET and other institutions, both national and international (including la Fundación Williams), the iBOL Argentina Fund has opened a call for research proposals with the aim of promoting the preservation of specimens of the country and region for DNA barcoding. The iBOL Argentina Fund is accepting proposals until January 31, 2016 and will finance up to 30 projects at a maximum of 50,000 pesos each. Researchers with accepted proposals are expected to participate in the special training workshop *Leading Labs Training Workshop for DNA Barcoding*, which will take place at the Museo Argentino de Ciencias Naturales Bernardino Rivadavia in 2016.

Feature

Image credit: Kate Pare

Identifying Patterns in Neotropical Collembola Diversity

Written by: Kate Pare and M. Alex Smith (University of Guelph, Canada)

Studying diversity patterns along elevation gradients allows for ecologists to infer how environmental factors influence species occurrence. However, elucidating diversity patterns can be challenging in groups which are difficult to identify by non-experts and in groups which contain morphologically similar but molecularly diverse species. Collembola, commonly known as springtails, are one such group affected by both of these taxonomic impediments.

Collembola are small hexapods found in the leaf litter, in soil, and on vegetation. These small and abundant invertebrates assist in decomposition, aid in fungal spore dispersal, increase soil quality and are important prey for both invertebrates and vertebrates. However, they are a challenging group to work with as their cryptic diversity makes them difficult to identify.

To study changes in Neotropical Collembola diversity along an elevation gradient we used estimates of both morphological and molecular diversity. Our study site, Volcán Cacao, located in the Área de Conservación Guanacaste (ACG), spans an elevational range of 1,500 m and contains three different forest types (dry forest, rainforest, and cloud forest). Collembola were collected as part of an ongoing standardised survey of leaf-litter arthropods on the three stratovolcanoes of the ACG. We estimated the diversity of Collembola using morphospecies, an interim proxy for species identification based on morphological characteristics. To estimate molecular diversity we used the barcode region of the COI mitochondrial gene and the nuclear 18S and 28S genes. These sequences were used to assign molecular operational taxonomic units (MOTUs) and to calculate phylogenetic diversity (PD) for both concatenated sequences (18S & 28S & COI) and COI sequences only.

"...they are a challenging group to work with as their cryptic diversity makes them difficult to identify."

We found no change in Collembola diversity measured as morphospecies richness, MOTU richness, or phylogenetic diversity when Collembola were analysed as a whole. Some evidence of relationships between diversity and elevation were found within the collembolan orders Entomobryomorpha, Poduromorpha, and Symphypleona; however ,these were not in the predicted direction.

"...this study highlights that each collembolan order differs in their sensitivity to changes in environmental factors..."

We were very surprised by these findings as Collembola are thought to be sensitive to desiccating conditions, and we expected to see an increase in Collembola diversity with elevation. We speculate that Collembola may be exploiting microhabitats which shelter them from the changing environmental factors along this environmental gradient. Furthermore, this study highlights that each collembolan order differs in their sensitivity to changes in environmental factors and that they should be considered separately in future work.

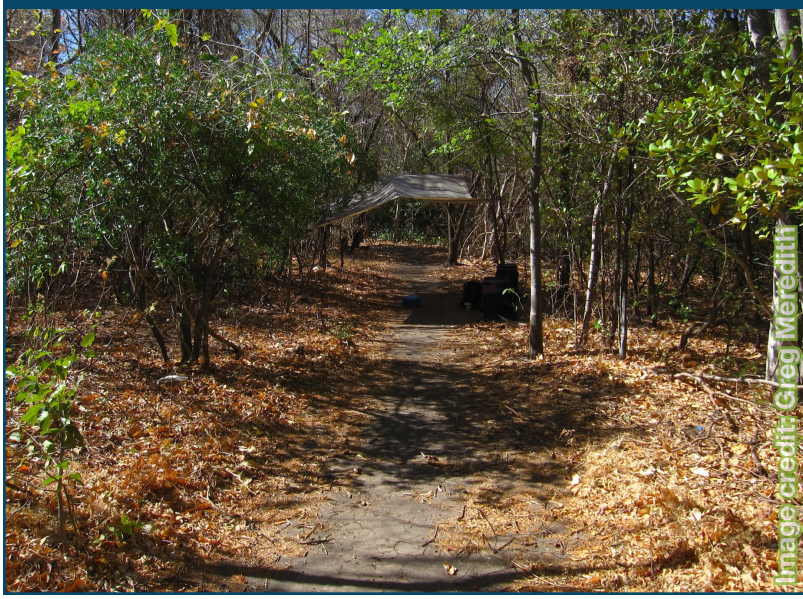
This study is a starting point for Collembola research along Volcán Cacao. The sequences generated from this research will be publically available on the Barcode of Life Data Systems, which will allow for Collembola to be monitored over time as climate change will bring warmer and drier conditions to this area.

This research was made possible by the ACG parataxonomists, Dan Janzen, Winnie Hallwachs, the Latornell graduate scholarship and travel grant, a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant, the Systematics Research Fund, the Leaders Opportunity Fund of the Canada Foundation for Innovation (CFI) and NSF DEB 0515699.

Cover photo by Greg Meredith.

Above: Hiking to our high elevation sample site.

Below: Our lowest elevation sample site.



Participants at the symposium on Biodiversity and DNA barcoding, 2015.

Biodiversity and DNA Barcoding Symposium in Norway

Written by: Ingrid Salvesen (Norwegian Biodiversity Information Centre, Norway) and Torbjørn Ekrem (NTNU University Museum, Norway, National coordinator of NorBOL)

In November this year, the Norwegian Biodiversity Information Centre (NBIC) and the Norwegian Barcode of Life Network (NorBOL) had the honour of inviting researchers, students, nature managers and everyone interested to the first national symposium on biodiversity and DNA barcoding in Norway. The two-day meeting, held in the city of Trondheim, hosted participants from Norway, Sweden, Germany, Finland, Canada, United Kingdom and the Czech Republic.

"The breadth and detail in the presentations were remarkable, and it was inspiring to learn about the steep increase in our knowledge of Norwegian biodiversity."

Our goal was to provide a venue for presentations and discussions of results from the Norwegian Taxonomy Initiative and national DNA barcoding efforts as well as give the participants some high-end research results from the international barcoding scene. Sixteen invited speakers were among the almost 100 participants that attended the meeting. Sixteen poster presentations decorated the lecture hall and encouraged discussions during the coffee breaks.

The Norwegian Biodiversity Information Centre (NBIC) is a public institution under the Norwegian Ministry of Education and serves as a national source of information on species and ecosystems in Norway. Its goal is to make up-to-date information on biodiversity widely available and easily accessible to society. NBIC coordinates the Norwegian Taxonomy Initiative as well as performs risk assessments of alien species and of threatened species, ecosystems and habitats. It also manages the national digital species map, registries of public observations (citizen science) and official name lists.

NORWEGIAN BIODIVERSITY INFORMATION CENTRE

Through the meeting, we heard excellent talks on BOLD (Sujeevan Ratnasingham), the Planetary Biodiversity Mission (Paul Hebert) and a number of interesting presentations on practical applications of DNA barcoding for biodiversity research and management. Natasha de Vere's presentation on barcoding of pollen from pollinators and Tomas Roslin's talk on the barcoding of Arctic food webs were impressive, as were all contributions on new biodiversity knowledge achieved through projects funded by the Norwegian Taxonomy Initiative.

The next meeting is planned for 2018, "just in time to celebrate NorBOL reaching its goal of barcoding 20,000 species along with the 10 year anniversary of the Norwegian Taxonomy Initiative."

We were taken on journeys from the very deep sea to the high mountains and were given peeks into the world of insects, earthworms, molluscs, bryophytes, fungi and past plant communities. The breadth and detail in the presentations were remarkable, and it was inspiring to learn about the steep increase in our knowledge of Norwegian biodiversity. It is obviously much more complex than at first sight!

And there is more to come, with new projects in the Norwegian Taxonomy Initiative starting every year and the barcode library of Norwegian species steadily growing.

We have received very positive feedback from participants at the meeting and were pleased to hear the enthusiasm concerning the work done by NorBOL and NBIC. This gives us encouragement to plan for the next meeting in 2018, which will hopefully take place just in time to celebrate NorBOL reaching its goal of barcoding 20,000 species along with the 10 year anniversary of the Norwegian Taxonomy Initiative.

Images by Åge Hojem, NTNU University Museum, CC-BY.

Speakers at the symposium. From left: Anders Hobæk, Christiane Todt, Marie Davey, Hans Tore Rapp, Frode Ødegaard, Inger Greve Alsos, Natasha de Vere, Endre Willassen, Kristian Hassel, Sujeevan Ratnasingham, Elisabeth Stur, Christer Erséus, Paul Hebert, Tomas Roslin. Tor Erik Brandrud and Gunn Paulsen were unavailable when the photo was taken.

DNA Barcoding in the Classroom: Investigating Fish Labeling

Written by: Rachel Hodgson, Tiana Waytuck, Matthew Morris, and Sean Rogers (University of Calgary, Canada)

During the fall semester of 2014 at the University of Calgary, our Molecular Ecology and Evolution class learned the skills necessary to answer ecological questions through the use of genetic markers. One such application of genetic markers is DNA barcoding, which aids in identifying samples of unknown origin.

As a class we used DNA barcoding to explore the question of whether or not the food we consume is correctly labeled. Specifically, we wanted to determine if the fish that we purchase from grocery stores, pubs, sushi restaurants, and other merchants is correctly labeled. This was the first study of its kind in Calgary.

"This project allowed us to participate in an ongoing food forensics and conservation question that has the potential to be a nation-wide concern..."

Hanner *et al.* (2011, DOI: 10.3109/19401736.2011.588217) conducted a similar study in five Canadian cities across British Columbia, Quebec, and Ontario. They determined that 41% of the fish samples they collected were incorrectly labeled. According to a study conducted by Marko *et al.* (2004, DOI: 10.1038/430309b), a commonly mislabeled fish species is red snapper (*Lutjanus campechanus*), which was mislabeled in 77% of their samples. Similarly, Hanner *et al.* (2011) found that 97% of their red snapper samples were mislabeled. Based on these findings we expected to find that at least a few of our samples would be mislabeled, with the greatest chance of mislabeling in our red snapper samples. Issues due to mislabeling of fish can include allergic reactions, improper nutrient intake, conservation concerns and incorrect pricing.

Our task was to work in pairs and collect samples of fish in the community from various retailers in order to determine whether or not the species had been properly labeled. Our class collected and preserved samples of fish in ethanol, which were then sent to the University of Guelph for sequencing. The sequences were uploaded to the Barcode of Life Data Systems, and we made inferences on labeling in accordance with the Canadian Food Inspection Agency's Fish List.

As this was a pilot study, sampling was not statistically rigorous – we were free to sample any fish product we liked. As a class we collected 18 samples which were marketed as follows: eight salmon/Atlantic salmon, three cod, two tuna, and one of each of halibut, red snapper, conger eel, short mackerel, and sockeye salmon. We found that 39% of these were mislabeled. A common species that we determined to be mislabeled was *Salmo salar* (salmon or Atlantic salmon), which accounted for 57% of our mislabeled samples and was most often identified by BOLD as *Oncorhynchus mykiss*, which is a species of trout. Our other mislabeled samples included short mackerel, cod, and red snapper, which were identified by BOLD as Indian mackerel, southern blue whiting, and tilapia, respectively.

A related issue we discussed involved legal but ambiguous fish labeling. We found that three of our samples, which were correctly labeled following the CFIA Fish List guidelines, were labeled so ambiguously that they could be a number of species. For example, our sample that was correctly labeled as "tuna" could actually be any one of 14 different species of tuna; only through BOLD could we identify the actual species. Legal but ambiguous labeling prevents consumers from identifying the exact species they are purchasing, which could have conservation implications.

This project allowed us to participate in an ongoing food forensics and conservation question that has the potential to be a nation-wide concern and partake in a study that is the first of its kind in Calgary. It introduced us to the method of DNA barcoding and the skills necessary for research in ecology. By doing our own data collection and making our own inferences, this project allowed us to see the potential we have as students in our undergraduate degree.

Thank you to the Barcode of Life, the University of Guelph, Amanda Naaum, and Dr. Hanner for their help and collaboration with this project!

Crowdfunding for DNA Barcoding Educational Initiative

Over the past three years, the School Malaise Trap Program has reached close to 15,000 elementary and secondary students across Canada, teaching them about the importance of biodiversity and showing them how DNA barcoding can help us in understanding the world around us. As part of the program, each participating school receives a Malaise trap to collect insects in their schoolyard for a specified two-week period. Following the collection period, the specimens are barcoded at the Centre for Biodiversity Genomics, and a report package of the results is sent to each school. Over 8,000 arthropod species have been collected by participants, with 1,288 of those species being new to the Barcode of Life Data Systems.

Although interest in the School Malaise Trap Program has grown rapidly since it began, our capacity remains unchanged. Each year we strive to involve as many schools as our resources allow, but, as resources diminish, we are asking for support from generous donors so that we may keep the program alive. From December 1, 2015 to January 12, 2016, the School Malaise Trap Program is hosting a crowdfunding campaign through the University of Guelph. Please consider donating and spreading the word to help us continue to offer this valuable educational opportunity. To learn more about our program goals and costs and to donate, click here.

Study site: Lake Anterne (Northern French Alps, 2063 m a.s.l)

Image credit: EDYTEM Laboratory (University Savoie Mont Blanc, France)

Environmental DNA Uncovers Past Biodiversity Changes

Written by: Johan Pansu (University Grenoble Alpes, France)

Reconstructions of past biological communities are traditionally inferred from natural archives. Lake sediments are widely used as, over time, they accumulate physical, chemical and biological material from the surrounding terrestrial environment via erosion and sedimentation. These studies rely mainly on macroremains and pollen, whose identification level and distribution can be variable.

Recently, a complementary approach based on sedimentary ancient DNA (*seda*DNA) emerged. The DNA of a variety of taxa, including plants and mammals, can be directly obtained from past sediments even in the absence of visible macrofossils. Environmental DNA (eDNA) metabarcoding seeks to identify multiple taxa from DNA contained in environmental samples in a single process by targeting short DNA fragments. We assessed the potential of this approach applied to eDNA preserved in lake sediments to provide reliable and novel information. We implemented this approach to investigate past biodiversity changes in response to human-induced environmental modifications around a high-elevation lake over the last 10,000 years.

"This study provided the first high-resolution reconstructed history of livestock farming over six millennia at the species level..."

Forty-seven slices were sampled from the sediment core of Lake Anterne (Northern French Alps), covering the last 10,165 years. As *seda*DNA is rare and there is an important risk of contamination by modern DNA, multiple DNA extractions and PCR replicates per sample (eight in total) were performed to maximise species detection and reveal potential sporadic contamination. Two primers pairs were used to characterise livestock farming history by targeting mammal DNA and plant communities around the lake.

First, we combined results from the *seda*DNA with a reconstruction of the frequency of erosive events to document the effects of human activities on erosion dynamics over the Holocene. The DNA-based reconstruction showed periods of pastoral activity with sheep and/or cowherds that mainly occurred in the Roman Period and Middle Age, corresponding to the most intense erosion phases. Specifically, during the Roman Period, the frequency of erosive events is the highest of the last 10,000 years despite a warm and dry climate which is supposed to be unfavourable for erosion crises. This study provided the first high-resolution reconstructed history of livestock farming over six millennia at the species level and evidenced the important and ancient role of past livestock farming on erosion dynamics and landscape shaping.

"Plant community trajectories over the Holocene were mostly related to soil evolution and pastoral activities..."

In a second study, we reconstructed plant community dynamics from DNA over the last 6,400 years. The high-quality data allowed reliable inferences about presence/absence patterns of plant taxa along the chronological record, the probability of false detection being always <5%. Eighty MOTUs that were strictly identical to referenced sequences in databases were detected, corresponding to 30 families, 39 genera and nine species. By comparing current species distribution data with combined data on past and present plant species assemblages and other paleoenvironmental proxies, we determined past habitat characteristics and assessed the relative impact of human activities, through pastoralism, and abiotic factors such as temperature and soil evolution.

Results showed an abrupt replacement of mountain forests dominated by *Pinus* trees and tall-herb communities (e.g. *Veratrum*, *Hypericum*) during the mid-Holocene by heathlands and grazed lands ~4,500 years ago, characterized by the presence of taxa specific of agro-pastoral systems (e.g. *Plantago*, *Alnus*). The mid-Holocene period corresponded to vegetation currently observed in relatively warmer, shaded and nutrient-rich environments, in comparison to vegetation over for the last 2,500 years. Plant community trajectories over the Holocene were mostly related to soil evolution and pastoral activities, highlighting the predominance of anthropogenic factors for their long-term evolution.

These studies highlight the relevance of *seda*DNA data, combined with other paleoecological proxies, to provide reliable information about past biodiversity changes in relation to human activities. Future improvements of this emerging approach, particularly taxonomic resolution, will open new avenues for research in paleoecology.

For more information on the studies described in this article, see DOIs: 10.1038/ncomms4211, 10.1111/mec.13136

Sediment core for environmental DNA analysis.

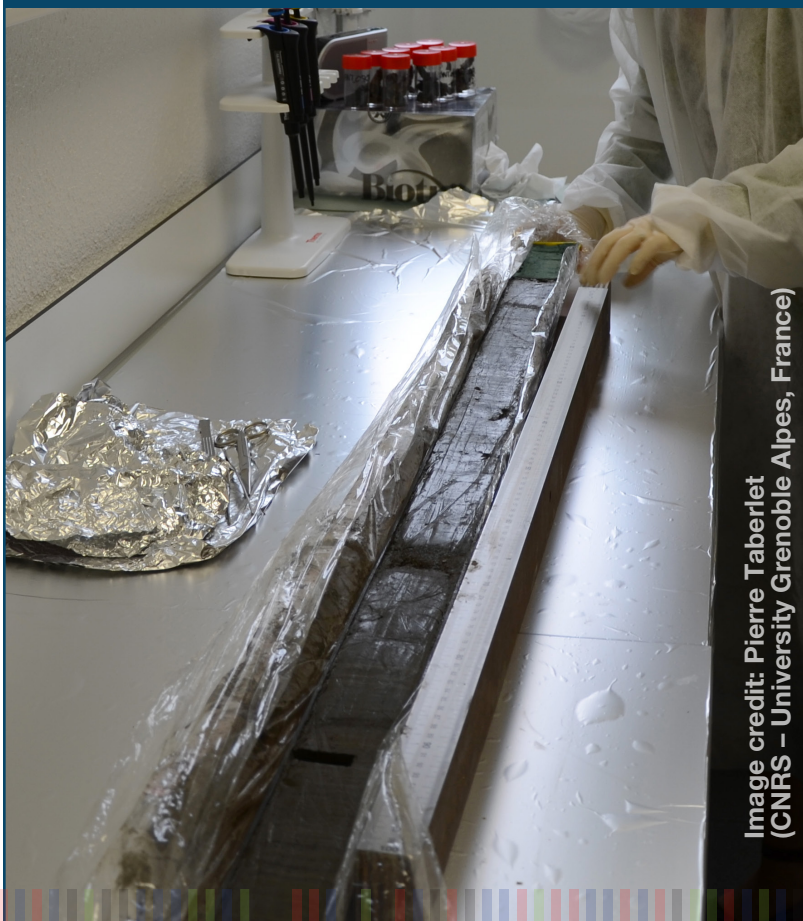


Image credit: Pierre Taberlet (CNRS – University Grenoble Alpes, France)

The Mauritanian Atlantic coast and continental shelf can be considered a threatened biodiversity hotspot.

© Wim C. Mullié

Monitoring Large Marine Vertebrates Through DNA Barcoding

Written by: Wim C Mullié, Oumar Bâ, Frédéric Marret (Mauritanian Ministry of Environment, Mauritania), Moulaye Mohamed Wagne, Abdellahi Samba Ould Bilal (Mauritanian Oceanographic and Fisheries Institute, Mauritania), Zein El Abidine Ould Sidaty (Diawling National Park, Mauritania), Jean-Luc Jung (laboratory BioGeMME, University of Brest, France), and Koen Van Waerebeek (Conservation and Research of West African Marine Mammals)

Biodiversity monitoring of marine habitats often targets areas of high species richness or of specific conservation concern. Both of these criteria apply to the coast of Mauritania due to the productive Canary Current upwelling system and growing maritime transport, along with the increasing use of the Mauritanian continental shelf by artisanal and industrial fisheries as well as oil and gas exploration and exploitation. In 2012, concerns for the risks of significant human impact on marine biodiversity led to the establishment of the Programme "Biodiversité, Gaz, Pétrole" (BGP; www.programmebgp.mr), spearheaded by the Mauritanian Ministry of Environment in close cooperation with the Ministry of Fisheries and the Ministry of Oil.

With the objective of establishing environmental baselines for several ecological and environmental parameters, field missions were a core activity of the programme. For the monitoring of large marine vertebrates, surveys of strandings were implemented every three months, focusing on the identification of species, quantification of individuals and diagnosis of possible causes of death (Mullié *et al.* 2013, DOI: 10.13140/RG.2.1.3988.9361).

During one such mission in November 2013, a field research team led by W. Mullié found a 4 m long, juvenile baleen whale stranded south of Chott Boul near Diawling National Park. The whale could not be collected, and its identity could not be determined in the field, partly due to advanced decomposition. Some of the rorquals, and especially their juveniles, including Bryde's (*Balaenoptera brydei*), Eden's (*B. edeni*) and Omura's whales (*B. omurai*), are difficult to distinguish morphologically, considering that their taxonomy remains unresolved and both intraspecific and interspecific variations are poorly described. Indeed, the complex phylogeny of cetaceans leads to new or formerly unrecognized species still regularly being described or resurrected.

With limited morphological evidence, DNA analyses can significantly boost the number of positively identified stranded cetaceans (Alfonsi *et al.* 2013, DOI: 10.3897/zookeys.365.5873); therefore, skin samples were sent for DNA barcoding. Despite poor sample quality due to decomposition, the sequencing of COI, *cytb* and the mitochondrial control region, for a total of 2,656 bp, revealed unambiguously that the specimen was an Omura's whale.

As the Omura's whale had never before been recognized in the Atlantic Ocean despite centuries of cetacean research and commercial whaling, this added a new species to an already rich catalog of Mauritanian cetaceans. While the range of the Omura's whale was previously thought to be restricted to the tropical and subtropical Western Pacific and Indian Oceans, a population was described off northwestern Madagascar in 2015, some 11,000 km away from Mauritania (Cerchio *et al.* 2015, DOI: 10.1098/rsos.150301). The stranded individual could theoretically have migrated to Mauritania from Madagascar but this is unlikely for a juvenile. Instead, it might belong to a hypothetical Eastern Atlantic population.

Omura's whale, never before recognized in the Atlantic Ocean despite centuries of cetacean research and commercial whaling, was not an obvious candidate ID for the stranded animal.

This discovery is a typical example of the benefits of systematic large-scale monitoring of biodiversity within strongly exploited ecosystems. Marine mammals are often considered relevant sentinels of the overall health of the world's oceans. Periodical field monitoring of the Mauritanian coast on a long-term basis, applying both morphology and DNA-based identifications of large marine vertebrates, should allow detection of any natural or anthropogenically-generated variations in species diversity and their relative composition, reflecting significant environmental changes. The Omura's whale might well become a flagship species for the conservation of Mauritania's marine wildlife in this ecologically and economically important region.

For more information about this research, see DOI: 10.1080/17451000.2015.1084424

Above: Specimen "MauBs", the first Omura's whale *Balaenoptera omurai* found in the Atlantic Ocean (November 3, 2013).

Below: ML tree-based identification of specimen MauBs. The sequences of three different markers (two partial coding sequences of COI and *cytb*, and the D-Loop) were determined and aligned with reference sequences, and a ML tree was constructed. The sequences of the specimen MauBs and of the *B. omurai* group in a monophyletic clade were supported by a maximum bootstrap of 1 (figure shows the example of the COI sequence).

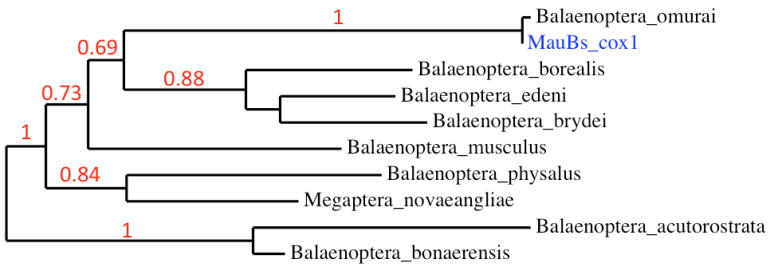


Image credit: Dirk Swanevelder

Metabarcoding of Pollen from a Historic Bee Collection

Written by: Annemarie Gous, Jurgens de Bruin, Connal Eardley, Dirk Swanevelder (Agricultural Research Council, South Africa), and Sandi Willows-Munro (University of KwaZulu-Natal, South Africa)

South Africa has one of the world's most diverse landscapes, with incredible plant richness and high pollinator biodiversity. The Agricultural Research Council (ARC) houses the National Collection of Insects with over 50,000 bee specimens collected during the past century. Given the vast diversity of plants and pollinators, and the threat of climate change on African biodiversity, it is important to study plant-pollinator interactions. DNA barcoding has been used successfully to identify the plants visited by bees using their pollen loads. Metabarcoding in palynology is still an emerging science; only recently did Alexander Keller and his team publish a full protocol for high-throughput barcoding of pollen using next-generation sequencing (NGS) of the internal transcribed spacer (ITS2, Keller *et al.* 2015, DOI: 10.1111/plb.12251). Most studies have only used fresh pollen or pollen preserved in ethanol. Access to the ARC's bee collection provided an opportunity to develop a method to study how floral choice has changed over time.

Pollen was taken from specimens of the solitary bee species, *Megachile venusta*. DNA barcoding was combined with Illumina NGS to identify samples spanning 93 years. Three genomic regions were studied: the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) and the *rbcL* gene. A database for Qiime or Miseq Reporter from publicly available sequences on NCBI was used to database the three barcode regions and retrain the Ribosomal Database Project (RDP) classifier in MacQiime 1.9.0. Twenty-two mixed-origin pollen samples were assessed. Samples dating from 1910 were successfully sequenced and classified. Species-level delimitation was possible for all genomic regions, with higher confidence at family level.

Historic bee collections provide access to study bee-plant interactions over time.

Universal barcodes like ITS identified both plant and fungal species. This opens interesting avenues for research in fungal-pollinator interactions.

Rarefaction analyses showed that, for ITS1 and ITS2, enough reads were sequenced to determine maximum species richness. For *rbcL*, only preliminary results were available, and more DNA must be sequenced to obtain maximum species richness. The ITS1 and ITS2 databases confidently classify sequences to genus level (c = 0.8), and *rbcL* enables family identification at the same confidence as ITS1 and ITS2.

ITS2 provided better resolution on a per sample basis with more identifications made with new additions to the databases. When comparing ITS2 taxonomic classifications from the database developed in this work with that of Sickel *et al.* (2015, DOI: 10.1186/s12898-015-0051-y), the latter made more classifications at the genus and species level. The ITS2 sequences used by Sickel *et al.* (2015) in their database were structurally verified and annotated as opposed to sequences taken from Genbank in this work. The preliminary *rbcL* data suggest that it identified families better than ITS1 and ITS2.

ITS barcoding also identified fungal sequences in variable amounts, with *Malassezia* found most frequently. This could be due to the samples being handled a lot and accumulating fungus through touch, in addition to fungus collected by the bees. The amount of fungi sequenced did not correlate with the age of the sample.

This is the first time that samples, spanning 93 years, have been used from a historic insect collection to study pollen origins using NGS and DNA barcoding techniques. The data showed that ITS2 could be used to identify pollen samples to genus level, and initial *rbcL* data suggest improved accuracy of these identifications.

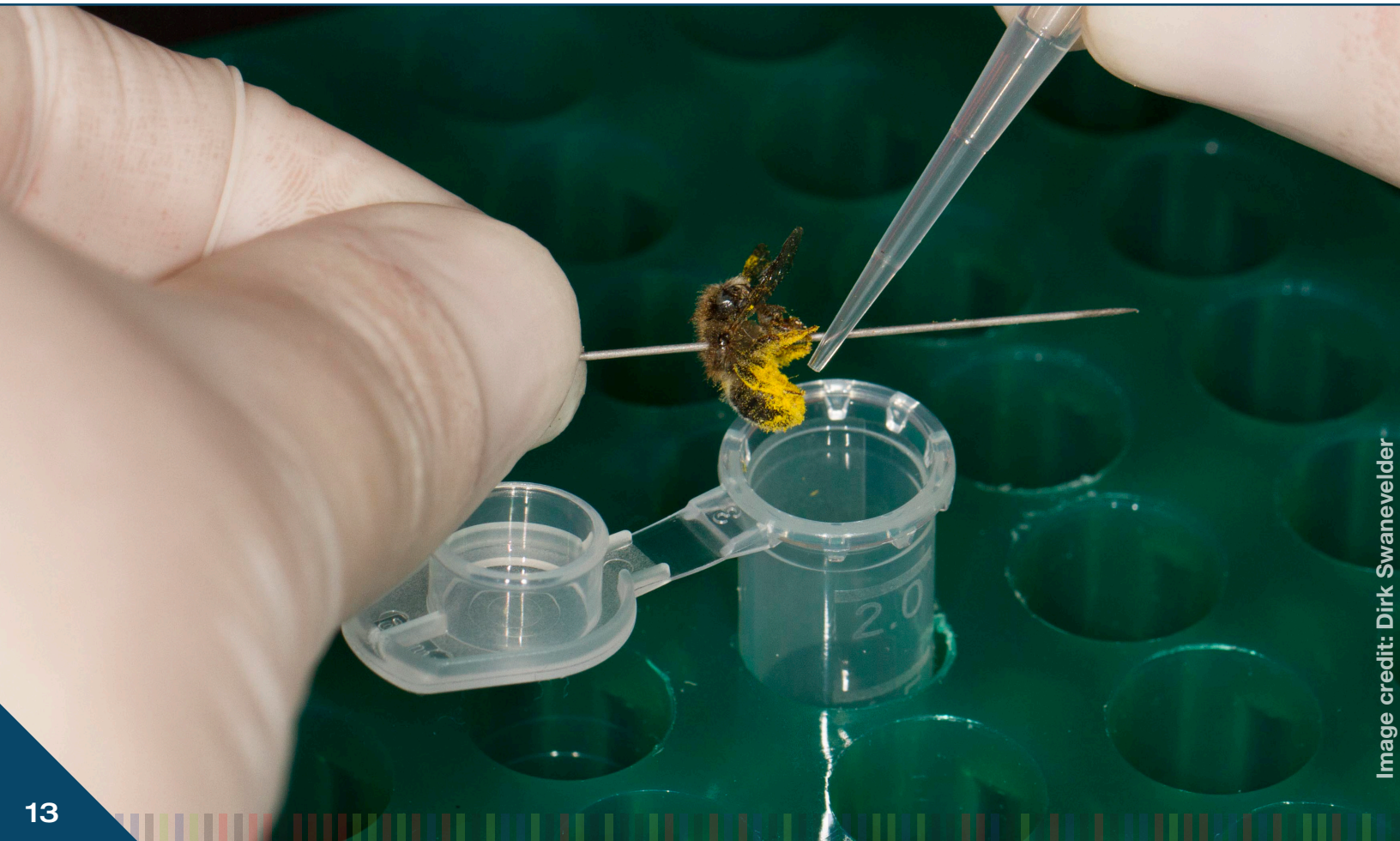


Image credit: Dirk Swanevelder

Qamar Abbas collecting insects in Kashmir hills.

Image credit: Saleem Akhtar

Pakistan Project Barcodes Over 5,000 Species

Written by: Muhammad Ashfaq (Centre for Biodiversity Genomics, Canada)

The Pakistan barcoding project has accomplished the milestone of generating 5K BINs. These BINs represent a comparable number of species from roughly 40,000 arthropods collected from diverse geographic locations, including plain agricultural lands and hilly reforested areas, from across the country. Insects comprised the majority of the collections and represented >96% of the total BINs from 12 insect orders; more than 80% of these BINs are not shared with other countries.

The project was launched in 2010 through collaboration between the Centre for Biodiversity Genomics (CBG) and the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan, with the aim of sequencing economically important insect species from crops and forests. The Higher Education Commission Pakistan provided an initial grant to cover the cost of specimen collection and front-end processing while the International Barcode of Life Project (iBOL) supported sequencing and databasing at CBG.

The scope of the Pakistan barcoding project was expanded in 2012 with funding support from the International Development Research Centre (IDRC) Canada, through CBG, which enabled DNA barcoding of human pests and disease vectors. The IDRC funding specifically supported studies on the distribution and genetic analysis of mosquitoes and head lice, the two major human parasites and disease vectors. The project has generated barcode data for notorious crop pests such as whiteflies, thrips, mealybugs, aphids, bollworms and grasshoppers, which have a high socio-economic impact. Barcoding of regional faunas of predominantly non-pests included antlions, predatory beetles, butterflies, odonates, pollinators and spiders, as well as extensive barcode coverage for the moth fauna (1,000 BINs). In addition, the Pakistan project contributed to CBG's Global Malaise Trap Program by running a Malaise trap in a biodiversity-rich zone, revealing temporal and spatial patterns of the regional biodiversity and recovering more than 2,200 BINs from a single site.

A significant amount of barcode data from crop pests and disease vectors has already been published in high-impact journals, and these data are available for reference for species identification and distribution analysis. Barcode analysis has also flagged cryptic diversity, highlighted endemic species and further resolved species distribution patterns over time and space.

Third National Meeting of MEXBOL

Written by: Manuel Elias Gutierrez and Martha Valdez Moreno (El Colegio de la Frontera Sur, Mexico)

From November 23-27 the Third National Meeting of the Mexican Barcode of Life Network was held at the Institute of Biology of the National Autonomous University of Mexico (UNAM), supported by the National Council of Science and Technology (CONACYT) and the National Commission for Knowledge and Use of Biodiversity (CONABIO). More than 120 academicians and students from more than 20 Mexican institutions were present. In addition, colleagues from Canada, USA, England, Netherlands, and Ecuador were in attendance. The opening talk, "A mission for planetary biodiversity", by Paul Hebert set a significant stage for this meeting in the 4th most biodiverse country of the world.

"As a result of the national, collective efforts of researchers and students... Mexico placed among the top 10 countries contributing to the International Barcode of Life project..."

CONACYT was represented by Verónica Bunge Vivier, Director of Thematic Networks. She recognized our network as one of the most successful and recommended to apply within the initiative of National Laboratories, as part of a further step to consolidate even more barcoding laboratories. CONABIO was represented by Liliana Lara, who showcased results of barcoding projects supported by them.

Manuel Elias Gutierrez gave insight into Mexico's role in the international barcoding efforts. As a result of the national, collective efforts of researchers and students interested in DNA barcoding, Mexico placed among the top 10 countries contributing to the International Barcode of Life project that just finished this year. Mario Rodríguez focused on the relevance of DNA barcoding of disease vectors. The next talk by Virginia León Règagnon gave an overview of the progress of the Google Barcode of Wildlife Project, in which Mexico was the first of six countries to reach the final phase, with implementation of techniques being passed on to government agencies responsible for border security. As part of this common effort, Monique Wesselink from the Netherlands Forensic Institute and Jesus Maldonado from the Smithsonian Institution provided an overview of forensics, conservation and DNA barcoding.

Of particular importance were 48 posters that showed different aspects of DNA barcoding in Mexico, with remarkable contributions by students of different academic levels.

"Among the most relevant outcomes is the interest to continue with this collective work and to continue with the construction of the national reference library as well as to integrate applications of these techniques."

In the following two days, several round tables were held to prepare different applications to be submitted and to look for potential funding stakeholders. This included a plenary to establish present and future commitments of the network. Among the most relevant outcomes is the interest to continue with this collective work and to continue with the construction of the national reference library as well as to integrate applications of these techniques.

Two workshops closed this meeting. The first one was on DNA barcoding of arthropods of medical and veterinary importance. The other was directed toward officials of governmental agencies (Federal Police (PF), Environmental Protection (PROFEPA), and Attorney General (PGR)) and focused on training on front-end processing for DNA barcoding.

In summary, after this fruitful meeting, we believe that Mexican academicians are ready to continue with Phase II of iBOL, and future results will provide a significant contribution by this hyperdiverse region of the world.

Virginia León Règagnon discusses the progress of the Barcode of Wildlife Project.

Gallery forest near the tropic of Capricorn.

Barcoding Australian Rainforest Plants to Help Conservation

Written by and images by: Alison Shapcott (University of the Sunshine Coast, Australia)

Rainforests are found across a wide expanse of Australia, from the top of the Australian tropics, inland from the Great Barrier Reef, through to the subtropics and all the way down to the temperate rainforest of Tasmania. This largely coincides with the regions where people like to live and grow their food; so some areas have had to compete with human needs and, as a result, have become highly fragmented. However, even before humans started clearing these forests, much of our rainforest estate was fragmented in a narrow band along the east coast due to the ancient drying of the Australian continent.

"...conservation assessments based on species diversity do not fully capture the phylogenetic diversity of these rainforests."

Australian flora has been long isolated and contains many unique species and genera. The rainforest plants show affinities to southern hemisphere forests that share past connections via Gondwanaland, but, in the north, they share taxonomic affinities with many tropical taxa that migrated to Australia via past land bridges to New Guinea. This history means that conservation assessments based on species diversity do not fully capture the phylogenetic diversity of these rainforests.

We are aiming to barcode the entire Australian rainforest estate to assist with conservation assessments and to provide a reference library for addressing a multitude of ecological questions, particularly related to plant-insect interactions. We have already barcoded 95% of the South East Queensland (SE Qld) subtropical rainforest estate (more than 800 species) and are well underway in the tropical rainforests, with representatives of all genera barcoded. We are making fresh collections for our library, with the vouchers being curated by the Queensland Herbarium, and many people have been inspired to assist us in these collecting efforts, including in quite remote locations.

Using detailed vegetation mapping records from the Queensland Herbarium along with their databases, we conducted phylogenetic diversity assessments of the rainforest in SE Qld according to biogeographic subregions. These assessments indicated which areas are phylogenetically distinct and need better representation in the Protected Area estate (Shapcott *et al.* 2015, DOI: 10.1371/journal.pone.0122164). We found that the northern rainforests of the region, near the tropic of Capricorn, are phylogenetically diverse and distinct within SE Qld but are very poorly conserved. We also found that rainforest diversity was not a function of the area of rainforest, either now or preclearing, and that some of the most diverse rainforest areas are quite well protected in the Protected Area estate.

"...the northern rainforests of the region, near the tropic of Capricorn, are phylogenetically diverse and distinct within SE Qld but are very poorly conserved."

We are now undertaking conservation assessments of phylogenetic diversity among different rainforest types in SE Qld and detailed assessments of specific areas identified from our subregion analysis, including the World Heritage area of Fraser Island. This is a real team effort involving collaboration among the Queensland Herbarium (Paul Forster, Bill McDonald, and Gordon Guymer), the Australian Tropical Herbarium / James Cook University (Darren Crayn and Craig Costion), the University of the Sunshine Coast, the Australian Museum (D. Faith), and the Kress Lab at the National Museum of Natural History, Smithsonian Institution.

Above: South East Queensland rainforest, on the Sunshine Coast.

Leaf cuts on a redbud tree, *Cercis canadensis*, by an unknown leafcutting bee, *Megachile* sp.

Image credit: Deb Chute

Investigating Leaf Preference among Leafcutting Bees

Written by: Scott MacIvor (York University, Canada)

There are over 350 species of wild bees in Toronto, and, in my research, I am interested in interpreting patterns in their diversity to support populations in urban planning and design. Discovering the 'bees' needs' includes matching floral resources but also identifying various nesting materials and locations required by different wild bee species. Many bee species are picky with regards to nesting materials and nesting location, while other species can be more flexible. This flexibility may provide them with an advantage when living in a city environment.

One group of bees I study are leafcutting bees (*Megachile*: Megachilidae). Leafcutting bees are unique among other wild bees in that the majority of them cut leaves with their mandibles from various trees, shrubs, wildflowers and grasses to build their nests. Leaf pieces are cut and flown back to the nest one at a time to partition and encase brood cells laid in a linear series, from the back of the nest to the front. Each brood cell is made of more than a dozen leaf pieces, and each nest can contain dozens of brood cells. Needless to say, it is a lot of work for the bees, and therefore having their preferred leaves located nearby would allow this building process to be more efficient.

"This missing detail in interpreting what bees need offers valuable information for conservation..."

To date, determining leaf preference of leafcutting bees has been difficult and dependent on morphological identification from leaf pieces or observation of known bees cutting leaves from known plants. As such, data on leaf preference are poor. This missing detail in interpreting what bees need offers valuable information for conservation by accounting for (potentially) limiting nesting material requirements.

To resolve this taxonomic issue, I used DNA barcoding to identify the leaves chosen by three leafcutting bee species that nest in 'bee hotels', which permitted easy access to their nests for study (MacIvor & Packer 2015, DOI: 10.1371/journal.pone.0122126). Nests were opened and one leaf piece from one cell per nest of the native *Megachile pugnata* and the introduced *Megachile rotundata* and *Megachile centuncularis* were examined. From 174 individual samples, 54 plant species were identified based on *rbcL* and ITS2 sequences.

"DNA barcoding revealed surprisingly high diversity in leaf preference by the three bee species."

DNA barcoding revealed surprisingly high diversity in leaf preference by the three bee species. Diversity in leaf preference was highest in introduced *M. rotundata*, followed by *M. centuncularis*, then native *M. pugnata*. There was significant overlap in leaf choice between the introduced bees. The leaves belonged to a variety of native and exotic species including trees, shrubs, and flowering plants, even one grass species. The exotic choices included some invasive species, including lamb's quarter (*Chenopodium album*), crown vetch (*Securigera varia*), purple loosestrife (*Lythrum salicaria*), and even dog-strangling vine (*Cynanchum rossicum*). By identifying these additional habitat needs of bees, landscape managers of urban plant communities have the potential to design areas that support more 'complete' pollinator habitat.

For more information about this study, please contact Scott MacIvor (jsmacivor@gmail.com)

A 'bee hotel' containing nesting tubes used by an assortment of cavity-nesting bees. In this design, a plastic PVC pipe encases 30 cardboard tubes of three different widths. The tubes are closed at the back and mimic plant stems or bore holes in wood that are used naturally by the bees.

Graphium sp.

Can Tropical Butterflies Cope with City Life?

Written by and images by: Kong-Wah Sing and John-James Wilson (University of Malaya, Malaysia)

Since 1990, the Federal Territory of Kuala Lumpur, Malaysia, has seen an 87% loss in "green" land, a 77% increase in the human population, and rapid urban sprawl across the outlying Klang Valley conurbation. Considering that Kuala Lumpur is found at the heart of a highly-threatened biodiversity hotspot, understanding the biodiversity "carrying potential" of the city habitats is critical, but so far has received little attention.

Butterflies react rapidly to environmental change due to their short generation time and high mobility. An estimated 20-40% of the butterfly species of Southeast Asia, many of them highly localized, are threatened with extinction due to urbanisation and deforestation across the region. To understand how well city parks can function as refuges for tropical butterflies, we surveyed butterflies at ten parks across Kuala Lumpur.

"An estimated 20-40% of the butterfly species of Southeast Asia, many of them highly localized, are threatened with extinction due to urbanisation and deforestation..."

The butterflies of Malaysia are diverse, and many are difficult to identify, especially "on the wing". Butterflies from the Hesperiidae and Lycanidae families, in particular, can be small, non-descript, and are often excluded from surveys. To circumvent this problem we used non-lethal tissue sampling, which has no effect on butterfly survival and reproduction, coupled with DNA barcoding to record the species diversity in each park. Accurate and precise identifications were made possible on the basis of DNA barcodes through comparison to a previously assembled DNA barcode reference library for butterflies of Malaysia from specimens in the Museum of Zoology at the University of Malaya.

"The lack of rare species in the parks in Kuala Lumpur, along with similar findings from Singapore and Hong Kong, indicates that tropical city parks are poor substitutes to natural habitats..."

Sixty butterfly species were found across the city parks, representing approximately 5% of the known butterfly fauna of peninsular Malaysia. However, almost all sampled butterflies were members of widely distributed, "common", species, suggesting that species with broad geographical distributions are those more likely to persist in cities. The lack of rare species in the parks in Kuala Lumpur, along with similar findings from Singapore and Hong Kong, indicates that tropical city parks are poor substitutes to natural habitats for maintaining populations of rare butterflies. Our study suggests that in order to promote butterfly diversity in tropical city parks, park managers should set aside areas of the parks as "unmanaged" or infrequently disturbed, semi-natural, habitat combined with a diverse planting scheme of native flowers in areas where management is necessary.

To further understand the patterns and determinants of urban butterfly diversity, we have recently completed butterfly surveys in city parks in Shenzhen, part of the Pearl River Delta of China, the world's biggest and most heavily populated megacity. It is clear from our chats with park users that urbanites would be very unhappy to see butterflies disappear from their neighbourhoods.

Above: Model of the city of Kuala Lumpur.

Below: Intensively managed plantings.

Malaise trap at Mpala Research Station, Kenya.

Image credit: Scott Miller

Exploring Global Arthropod Diversity

Written by: Kate Perez (Centre for Biodiversity Genomics, Canada)

Since 2012, the Centre for Biodiversity Genomics (CBG) has worked in collaboration with various international contributors to map out detailed information on terrestrial arthropod communities around the world through the Global Malaise Trap Program (GMP). To date, 73 sites from 35 countries have participated in GMP with traps deployed in a diversity of ecosystems ranging from Arctic tundra to tropical rainforests. The standard methods of Malaise trapping and DNA barcoding make it possible to carry out large-scale sampling programs and enable a time- and cost-efficient approach for biodiversity assessments.

"...73 sites from 35 countries have participated in GMP with traps deployed in a diversity of ecosystems ranging from Arctic tundra to tropical rainforests."

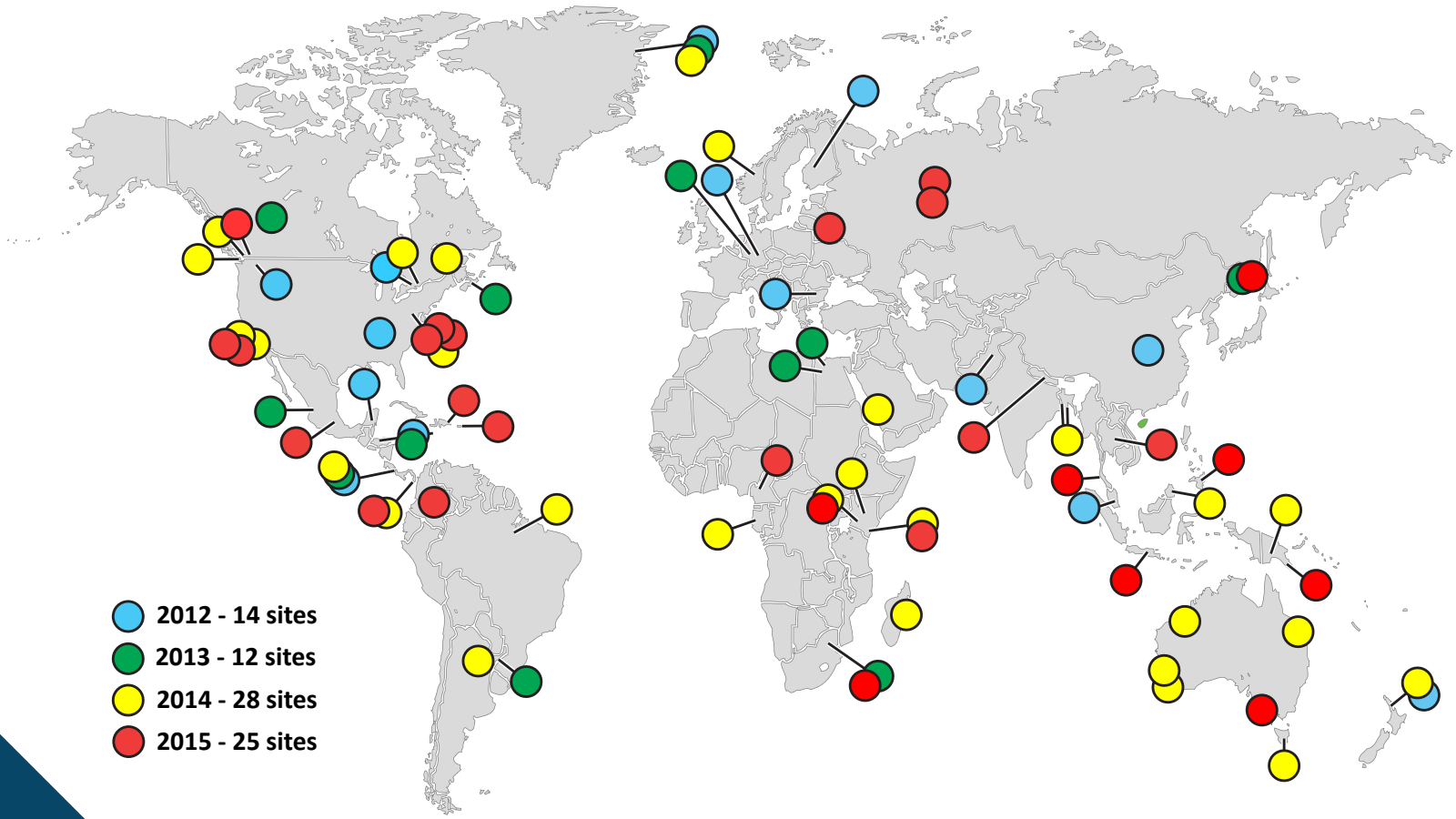
Partners are provided with a standard sampling kit complete with a brand new Malaise trap and enough bottles to collect weekly during the season(s) of arthropod activity. All samples are returned to CBG for processing through the Sanger-based analytical pipeline, and the Barcode Index Number (BIN) algorithm is utilized to determine if each individual specimen belongs to a new or existing barcode cluster.

Much of the process is automated, and minimal time and reagents are put into the initial screening. Only the subset of new BINs to BOLD undergoes complete processing; samples are retrieved and bi-directionally sequenced to ensure high quality barcodes, and up to five voucher representatives of each new BIN are imaged. This BIN-based approach of processing specimens can be completed for roughly one fifth of the cost of standard workflows, and this is what makes the Malaise protocol unique.

Malaise samples from 40 sites across 27 countries have been processed to date, with an average of 22 samples per location. The number of specimens collected at each site varied from less than a thousand to over 63,000. Rates of species accumulation varied greatly among different GMP locations. For instance, multiple years of sampling at the Zackenberg Research Station in Greenland collected nearly 20,000 specimens but only 263 BINs. On the other hand, only a very small fraction of the diversity has been captured in sites such as the Gombak Field Studies Centre in Malaysia and Andasibe-Mantadia National Park in Madagascar (which collected over 6,000 BINs each), according to their steep accumulation curves.

A total of 795,119 specimens have been collected through the program, providing some 77,000 BINs and a species accumulation curve that shows no signs of saturation. A different BIN is encountered roughly every 10 specimens, and nearly half of the BINs are represented by just a single individual. Over 60% of specimens captured were flies (Diptera) followed by wasps, ants, and bees (Hymenoptera), which comprised 14%. Butterflies and moths (Lepidoptera), beetles (Coleoptera), and true bugs (Hemiptera) each comprised around 5-7% of the total. Less abundant groups also provided considerable diversity with another 36 orders represented in the remaining 5% of collected taxa.

Through the help of numerous collaborators, GMP has accumulated a substantial amount of occurrence data across the planet and has begun the long process of constructing barcode reference libraries for terrestrial arthropods globally. Interpretations of emerging trends are highly tentative due to undersampling and seasonality issues. Much remains unidentified, but an effective network of experts is continuously being built to cooperatively increase taxonomic resolution. Ultimately, the project may allow for the exploration of long-standing hypotheses in biodiversity science, most of which were developed with plants and vertebrates, and more accurately develop global species estimates for terrestrial arthropods.



Endorsement by the Convention on Biological Diversity

Written by and image by: Alex Borisenko (Centre for Biodiversity Genomics, Canada)

The nineteenth meeting of the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA 19) to the Convention on Biological Diversity (CBD) brought together 500 delegates and observers in Montreal, Canada on November 2–5, 2015. It dealt with technical and strategic matters related to the recent findings of the fourth edition of the Global Biodiversity Outlook (GBO 4) and the preparation for the upcoming Thirteenth Conference of the Parties (COP 13). The meeting provided a venue to highlight recent advances in DNA barcoding during two side events and at the joint kiosk, kindly facilitated by Ms. Junko Shimura from the CBD Secretariat (SCBD).

DNA barcoding was mentioned in the document on Scientific and Technical Needs Related to the Implementation of the Strategic Plan for Biodiversity 2011-2020 (UNEP/CBD/SBSTTA/19/3), introduced by the SCBD. It emphasized the importance of supporting the Global Taxonomic Initiative (GTI) as the foundation for building the knowledge base needed to implement the global Strategic Plan for Biodiversity 2011-2020 and to make relevant policy decisions.

In the discussion that followed, Netherlands and Germany cautioned against the specific mention of DNA barcoding and other tools; however, the Parties were generally in favour of promoting the GTI and relevant instruments to help improve biodiversity knowledge. France and Belarus endorsed GTI implementation, and South Africa underscored the importance for DNA barcoding capacity building and academic training to aid in species identification.

In the end, DNA barcoding was referenced in Paragraph 5(g) of SBSTTA 19 Recommendation XIX/2 that encourages Parties "*To support the development, with the assistance, as appropriate, of the international barcode of life network, of DNA sequence-based technology (DNA barcoding) and associated DNA barcode reference libraries for priority taxonomic groups of organisms, to promote the application of these techniques for the conservation and sustainable use of biodiversity, and to support related capacity-building activities, including relevant academic training, as appropriate, further to the Strategic Actions 3 and 4 of the capacity-building strategy for the Global Taxonomy Initiative*".

This recognition is an important milestone for the global barcoding community, signifying that DNA barcoding has matured not only as a research discipline but also as a widely known and accepted tool to help inform international policymaking.

Modeling Plant Extinctions and Arthropod Coextinctions

Written by: Carlos Garcia-Robledo, W. John Kress, Terry L. Erwin, Charles L. Staines (Smithsonian Institution, United States), and Evert Lindquist (Canadian National Collection of Insects, Arachnids and Nematodes, Canada)

We are losing species at an unprecedented rate. One of the factors generating this mass extinction is global warming. If CO_2 emissions continue at the current rate, with an increase of 3°C in the next century, it is expected that one out of six species on earth will be at risk of extinction (DOI: 10.1126/science.aaa4984).

This scenario could be further exacerbated; as evolutionary biologist Dan Janzen proposed, "What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions". As species become extinct, this may generate the extinctions of other organisms that depend on the species that disappeared. This process is called coextinction. Unfortunately, we still don't understand the magnitude at which primary extinctions, initially generated by global warming, will propagate through interaction networks generating coextinctions.

DNA barcoding now allows scientists to explore one of the most pervasive sources of biodiversity loss, species coextinctions.

One of the challenges to estimate coextinction rates is to determine interactions among organisms. The use of DNA barcodes represents a unique tool to identify taxa with poorly known taxonomy, as well as their interactions with other organisms.

We are performing this project along the La Selva-Braulio Carrillo National Park, Costa Rica. This is the highest elevational gradient of continuous forest in Central America.

To estimate extinction-coextinction rates under projected global warming, we are reconstructing interactions among plants from the order Zingiberales, their insect herbivores, rolled-leaf beetles (genera *Cephaloleia* and *Chelobasis*), and phoretic mites that hitchhike on rolled-leaf beetles to move between host plants. We are recording these interactions along the elevational gradient, from 50 m to 2000 m.

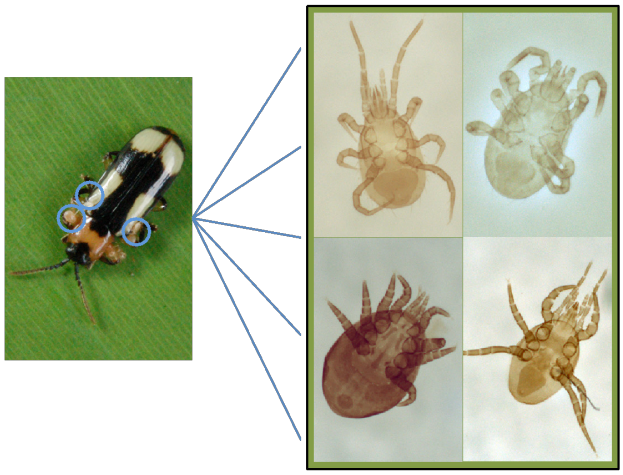
We assembled a DNA barcode library containing *rbcL* and ITS2 sequences from all potential Zingiberales host plants. This reference library was used to identify plant DNA extracted from insect gut contents. Combining DNA barcode identifications and field records, we now know the diets for all known rolled-leaf beetles present along the elevational gradient (at least 36 beetle species).

To assemble interactions between rolled-leaf beetles and phoretic mites, we are removing thousands of mites from each rolled-leaf beetle species present at different elevations. After DNA extraction, exoskeletons are mounted on slides for further taxonomic identification.

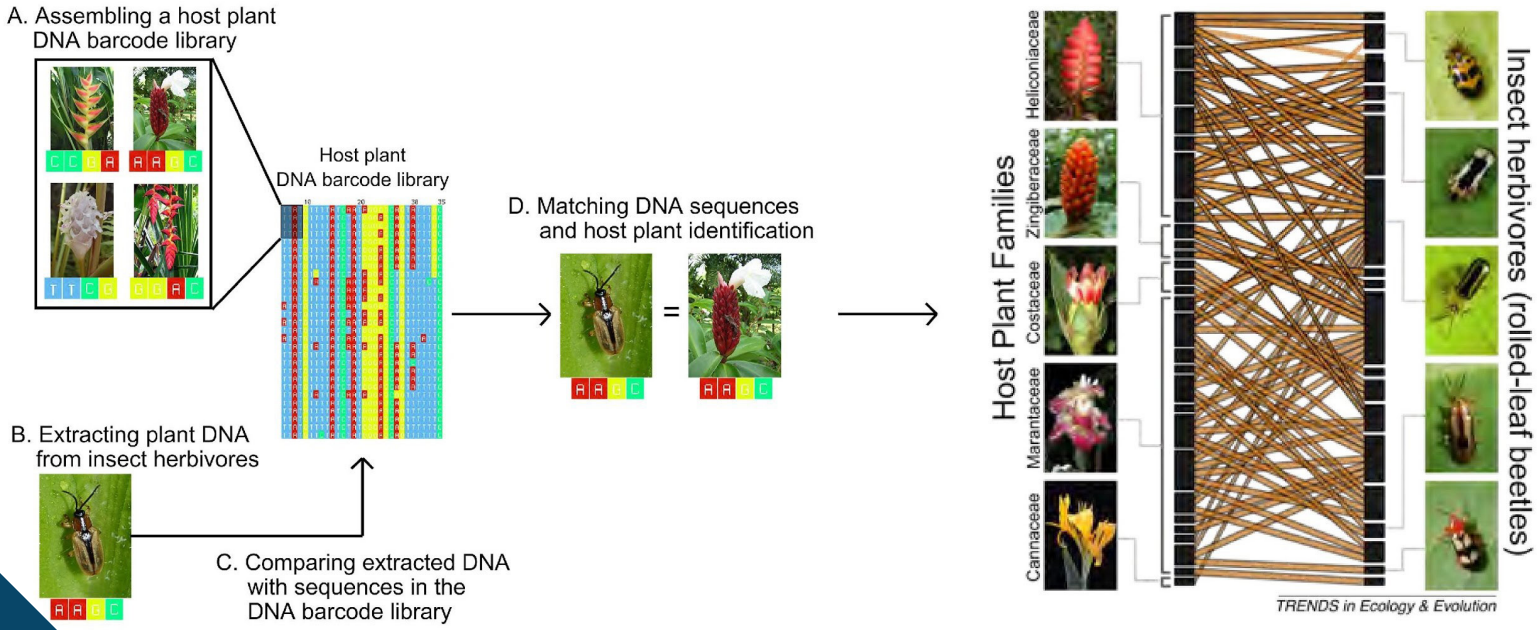
The main objective of this ongoing project is to combine global warming models for our study locality and DNA barcode-based interaction networks to model cascades of extinctions. Models will estimate, under global warming scenarios, coextinctions from host plant to insect herbivore and other arthropods such as phoretic mites. DNA barcoding now allows scientists to explore one of the most pervasive sources of biodiversity loss, species coextinctions.

For more information on the results discussed in this article, see DOIs: 10.1371/journal.pone.0052967, 10.1016/j.tree.2014.10.008, 10.3897/zookeys.477.8220, 10.1111/bij.12115

Using the DNA barcode COI, we are identifying molecular operational taxonomy units (MOTUs) of phoretic mites associated with each rolled-leaf beetle species along the La Selva – Braulio Carrillo elevational Gradient in Costa Rica. We are sampling beetle-mite interactions from the tropical rain forest at La Selva Biological Station (50 m elevation) to montane forests in Braulio Carrillo National Park (2000 m elevation). In preliminary analyses based on 919 COI sequences, we recorded 92 MOTUs. Phoretic mites include individuals from the genera *Antennocheles* (Antennochelidae), *Hispiniphis* (Melicharidae) and *Lasioseius* (Blattisociidae).



Identification of insect herbivore host plants using DNA barcodes. We assembled a DNA barcode library containing *rbcL* and ITS2 sequences from all potential Zingiberales host plants. After amplifying the DNA barcodes *rbcL* and ITS2 from insect gut contents, we identified insect diets using the reference library. Combining molecular host plant identifications and field records, we identified rolled-leaf beetle – Zingiberales host plant interactions along the highest elevational gradient of continuous forest in Central America.



Research

Top 10 DNA Barcoding Publications 2015

Measured using Publish or Perish (Jan-Dec) Metrics are largely based on Google Scholar ranking and journal access statistics.

1. Li X, Yang Y, Henry RJ, *et al.* (2015) Plant DNA barcoding: from gene to genome. *Biological Reviews* 90(1): 157-66. DOI: 10.1111/brv.12104

2. de Vargas C, Audic S, Henry N, *et al.* (2015) Eukaryotic plankton diversity in the sunlit ocean. *Science* 348(6237): UNSP 1261605. DOI: 10.1126/science.1261605

3. Levy SF, Blundell JR, Venkataram S, *et al.* (2015) Quantitative evolutionary dynamics using high-resolution lineage tracking. *Nature* 519(7542): 181-6. DOI: 10.1038/nature14279

4. Martinsson S, Rota E, Erseus C (2015) Revision of *Cognettia* (Clitellata, Enchytraeidae): re-establishment of *Chamaedrilus* and description of cryptic species in the *sphagnetorum* complex. *Systematics and Biodiversity* 13(3): 257-77. DOI: 10.1080/14772000.2014.986555

5. Carvalho DC, Palhares RM, Drummond MG, *et al.* (2015) DNA Barcoding identification of commercialized seafood in South Brazil: A governmental regulatory forensic program. *Food Control* 50: 784-8. DOI: 10.1016/j.foodcont.2014.10.025

6. Handley LL (2015) How will the 'molecular revolution' contribute to biological recording?. *Biological Journal of the Linnean Society* 115(3): 750-66. DOI: 10.1111/bij.12516

7. Schmidt S, Schmid-Egger C, Moriniere J, *et al.* (2015) DNA barcoding largely supports 250 years of classical taxonomy: identifications for Central European bees (Hymenoptera, Apoidea *partim*). *Molecular Ecology Resources* 15(4): 985-1000. DOI: 10.1111/1755-0998.12363

8. Baniecki ML, Faust AL, Schaffner SF, *et al.* (2015) Development of a single nucleotide polymorphism barcode to genotype *Plasmodium vivax* infections. *PLoS Neglected Tropical Diseases* 9(3): e0003539. DOI: 10.1371/journal.pntd.0003539

9. Leray M, Knowlton N (2015) DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences* 112(7): 2076-81. DOI: 10.1073/pnas.1424997112

10. Lamendin R, Miller K, Ward RD (2015) Labelling accuracy in Tasmanian seafood: An investigation using DNA barcoding. *Food Control* 47: 436-43. DOI: 10.1016/j.foodcont.2014.07.039

Credits and Contributions

Editors: Dirk Steinke, Emily Berzitis
Layout: Suz Bateson

The Barcode Bulletin owes its success to the valuable contributions of researchers and enthusiasts within the global DNA barcoding community. If you wish to contribute please contact us at barcodebulletin@gmail.com.