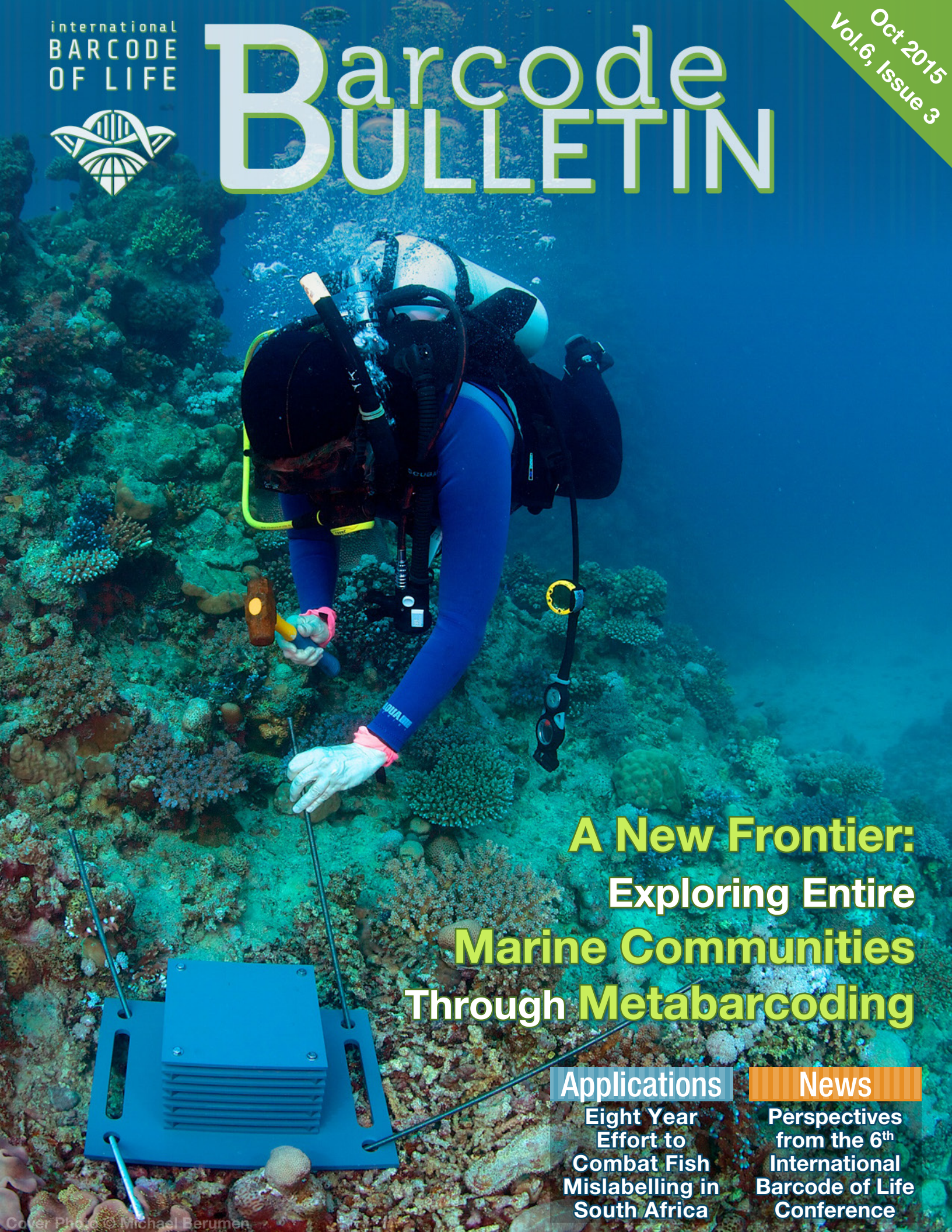
international BARCODE OF LIFE

Barcode BULLETIN

Oct 2015
Vol.6, Issue 3



A New Frontier: Exploring Entire Marine Communities Through Metabarcoding

Applications

Eight Year Effort to Combat Fish Mislabelling in South Africa

News

Perspectives from the 6th International Barcode of Life Conference

Cover Photo © Michael Berumen

Welcome to the Fall 2015 issue of the iBOL Barcode Bulletin.

The 6th International Barcode of Life Conference in Guelph closed its doors about two months ago. It's now time to look back at this record breaking gathering of experts in biodiversity science. Two contributions in this issue will provide you with summaries from different perspectives and a number of articles showcase topics that were presented at the conference.

This issue could be titled "of lice, lichens and larvae" but this would only cover a fraction of the topics considered. We tried to include many of the prize winners at the conference (see column on the right) in this issue to showcase their work and more will be featured in the next issue. Once again I would like to thank all contributors for their willingness to share their findings with the Barcode Bulletin community.

Dirk Steinke
Editor-in-chief

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Conference Prize Winners

Fifteen prizes were awarded to graduate students and postdoctoral fellows at the 6th International Barcode of Life Conference, recognizing excellence in research and in socio-economic applications.

Graduate Students - Oral Presentations

Dorcas Lekganyane, African Centre for DNA Barcoding, South Africa

Kristiina Mark, University of Tartu, Estonia

Kate Pare, University of Guelph, Canada

Jennifer Hawkins, National Botanic Garden of Wales and Cardiff School of Pharmacy and Pharmaceutical Sciences, UK

Kong-Wah Sing, University of Malaya, Malaysia

Claire Beet, University of Waikato, New Zealand

Graduate Students - Poster Presentations

R. Gabriela Aguilar-Velasco, Instituto de Biologia UNAM, Mexico

Justin Bernstein, Villanova University, USA

Iliana Bista, Bangor University, UK

Saloni Malik, University of Delhi, India

Thomas Royle, Simon Fraser University, Canada

Postdoctoral Fellows - Oral Presentations

Kristy Deiner, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Switzerland

Donna-Maree Cawthorn, Stellenbosch University, South Africa

Postdoctoral Fellows - Poster Presentations

Valentina Todisco, University of Vienna, Austria

Seikoh Saitoh, Tropical Biosphere Research Center, University of the Ryukyus, Japan

Larger marine species from ARMS, such as this terebellid worm from the Red Sea (Jordan), can be barcoded by traditional methods.

Feature

Image credit: Matthieu Leray

Metabarcoding the Ocean

Written by: Matthieu Leray and Nancy Knowlton (National Museum of Natural History, Smithsonian Institution, United States)

Understanding how many species live in the ocean, where they live, and how they interact and respond to environmental stressors is of great importance in the context of global change. Yet, these fundamental questions have proven extremely challenging to answer using traditional methods because many marine species are hard to identify except by experts. Even barcoding of individual organisms can be difficult because most marine species are microscopic and they vary enormously in abundance from very rare to extremely numerous. This has limited the use of barcoding for large-scale inventories and ecological studies looking at whole communities.

High-throughput sequencing makes it possible to survey marine organisms that are ignored by traditional approaches.

This is rapidly changing now, as High-Throughput Sequencing (HTS) technologies open up new frontiers in marine biodiversity science by making it possible to read DNA barcodes for hundreds of specimens at a time. This approach, termed DNA metabarcoding, holds considerable promise for rapid and more comprehensive biodiversity monitoring of the ocean on a large scale, particularly when combined with standardized sampling methods.

In recent studies conducted in the US, Belize, Panama, Jordan and Saudi Arabia, we have characterized complex communities of metazoans from Autonomous Reef Monitoring Structures (ARMS). ARMS can be thought of as underwater condominiums that mimic the complex three-dimensional structures of oyster and coral reefs, communities that are otherwise difficult to sample in a consistent manner. After the ARMS are collected, we follow a standardized protocol that ensures preservation of high molecular weight DNA and prevents cross-contamination. We individually barcode the larger organisms (>2 mm) using established approaches and metabarcode all the other community members (sessile organisms and two size classes of smaller unattached organisms) by metabarcoding of a short fragment of the mitochondrial Cytochrome Oxidase Subunit 1 gene (COI). For the metabarcodes, we use a highly conservative sequence analysis pipeline to retain high quality COI reads only.

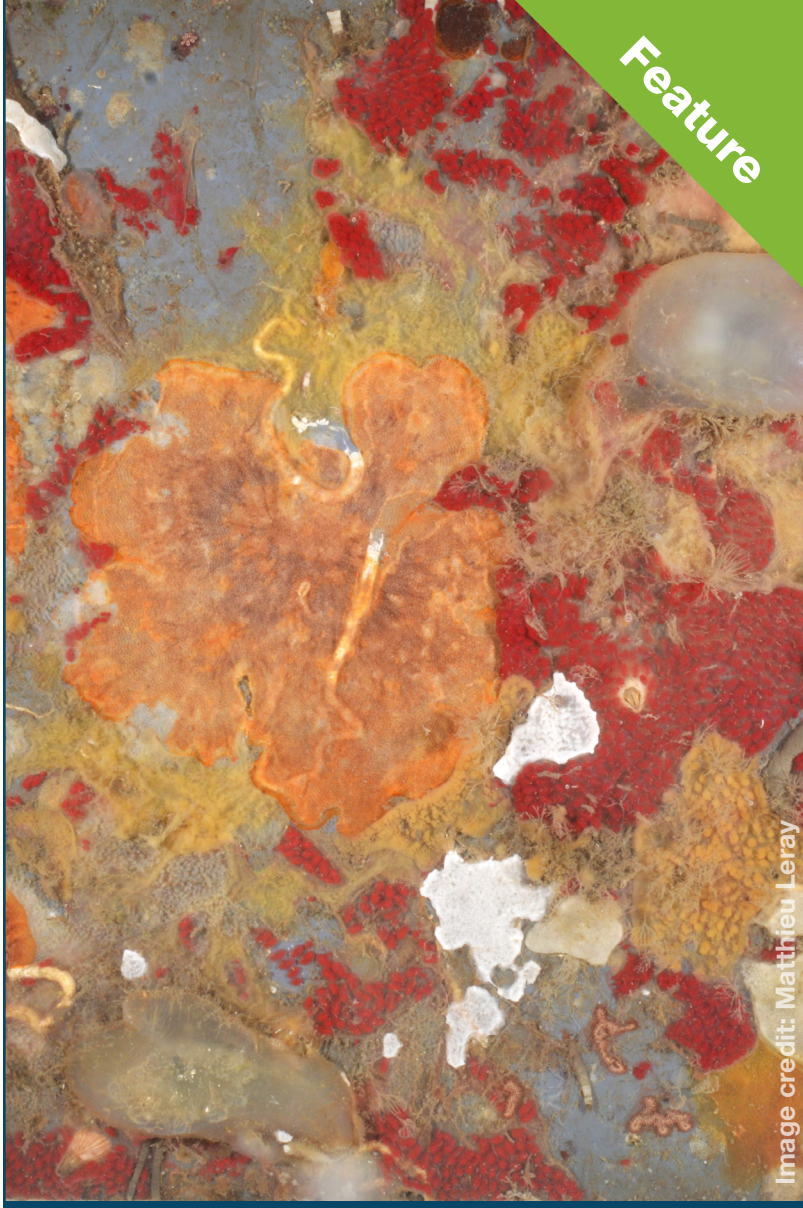
On oyster reefs in Florida and Virginia (USA), we left 18 ARMS (9 at each location in three clusters of three) on the seafloor for six months, during which time they were colonized by local bottom-dwelling species. Out of approximately 1 million sequences, we detected a total of 2,179 Operational Taxonomic Units (OTUs), only 10.9% of which matched sequences in public databases.

Metabarcoding provides reliable information on what species are present and their relative importance as measured by biomass.

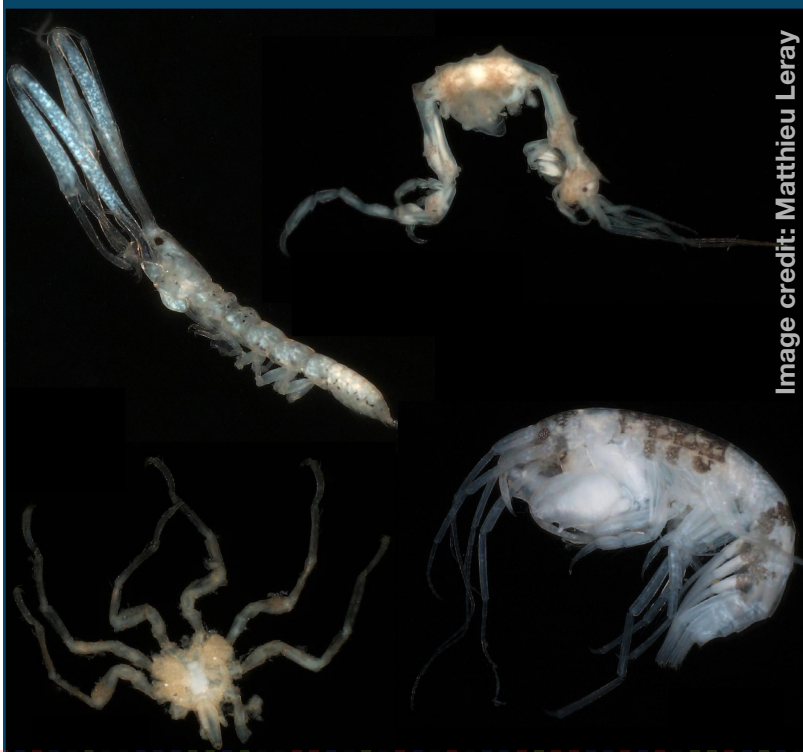
Communities of animals measuring between 500 µm and 106 µm represented more than two thirds of the total diversity, which illustrates that conventional surveys that only consider larger organisms fail to capture most living forms. There was a clear latitudinal gradient in diversity from temperate Virginia to sub-tropical Florida, but small organisms showed more overlap between Florida and Virginia than did larger ones. Finally, we found evidence for final-scale spatial structuring, as communities separated by 2 m were more similar in terms of community composition than communities that were 100 m apart. These findings were validated using direct comparisons to traditional specimen-based assessments of diversity, which showed that HTS successfully captured diversity and gave useful information on relative abundance of the taxa.

There is an urgent need to move from the study of a few indicator groups to whole communities to better predict how human activities impact all forms of life. DNA metabarcoding of standardized samples is a powerful approach to study how benthic communities change across time and space. Implemented on a range of marine habitats and on a global scale, it could help build the first baseline map of patterns of marine diversity across the metazoan tree of life.

For more information about this research, see DOI: 10.1073/pnas.1424997112



Sessile community (above) and tiny crustaceans (500 µm - 2 mm; below) from ARMS deployed in Florida and effectively surveyed using metabarcoding.



Sixth International Barcode of Life Conference

Written by: Sarah Adamowicz and Meg Fritzsche (Biodiversity Institute of Ontario, Canada)

From Aug 17-21, 2015, delegates gathered in Guelph, Canada for the 6th International Barcode of Life Conference. This was the largest meeting to date in this biennial conference series, with 601 delegates representing 51 countries in attendance! With such increasing participation and interest, Paul Hebert's vision for an international mega-project that he outlined in his plenary talk — termed the Planetary Biodiversity Mission — may be realized.

Themed "Barcodes to Biomes", the scientific program featured plenary talks by internationally renowned researchers, diverse parallel sessions, and a dedicated poster session. Emerging research trends apparent from this conference included an expanded emphasis upon diverse socio-economically important applications of DNA barcoding, increasing usage of DNA barcoding and metabarcoding for ecological and evolutionary research, and more studies investigating species interactions across multiple trophic levels. The special workshop hosted by the Canadian Food Inspection Agency also indicates how interest in DNA barcoding has firmly expanded beyond academia. You may read more about research trends apparent among the conference presentations here.

"a special plenary session entitled "State of Biodiversity" ... invited reflection and renewed commitment to study and protect biodiversity."

All conference abstracts have been published in a special issue of the journal *Genome* and are freely available online here. *Genome* also welcomes submissions of full articles for two post-conference thematic special issues (due date Nov. 30, 2015). For further information, please contact the Lead Guest Editor (Sarah Adamowicz: sadamowi@uoguelph.ca).

Among the highlights of the conference was a special plenary session entitled "*State of Biodiversity*", hosted at the lovely River Run venue in downtown Guelph. Experts on biodiversity in different biomes delivered thoughtful talks that provided an overview of the current state of biodiversity on our planet. Their presentations invited reflection and renewed commitment to study and protect biodiversity. Plenary presentations from the main scientific program as well as from the two special sessions are available for viewing here.

Congratulations to the 15 winners of the student and postdoctoral prizes (see the list of prize winners and learn about their research here). Thank you very much to the established researchers who kindly served as evaluators; this was certainly not an easy task due to the impressive quality of numerous presentations. We greatly look forward to future contributions by all up-and-coming members of our research community.

In association with the conference, staff members of the Biodiversity Institute of Ontario worked together with an international team of taxonomic experts to conduct a bioblitz of the ***rare*** **Charitable Research Reserve**. The team rapidly published the results by the end of the conference week! See the many-authored publication here (DOI: 10.3897/BDJ.3.e6313). This effort resulted in >1,000 new species records for the reserve and generated a large collection of digitized, barcoded specimens available for further scientific study. This impressive effort also showcased how efficiently a dedicated team of collaborating experts can advance knowledge of biodiversity.

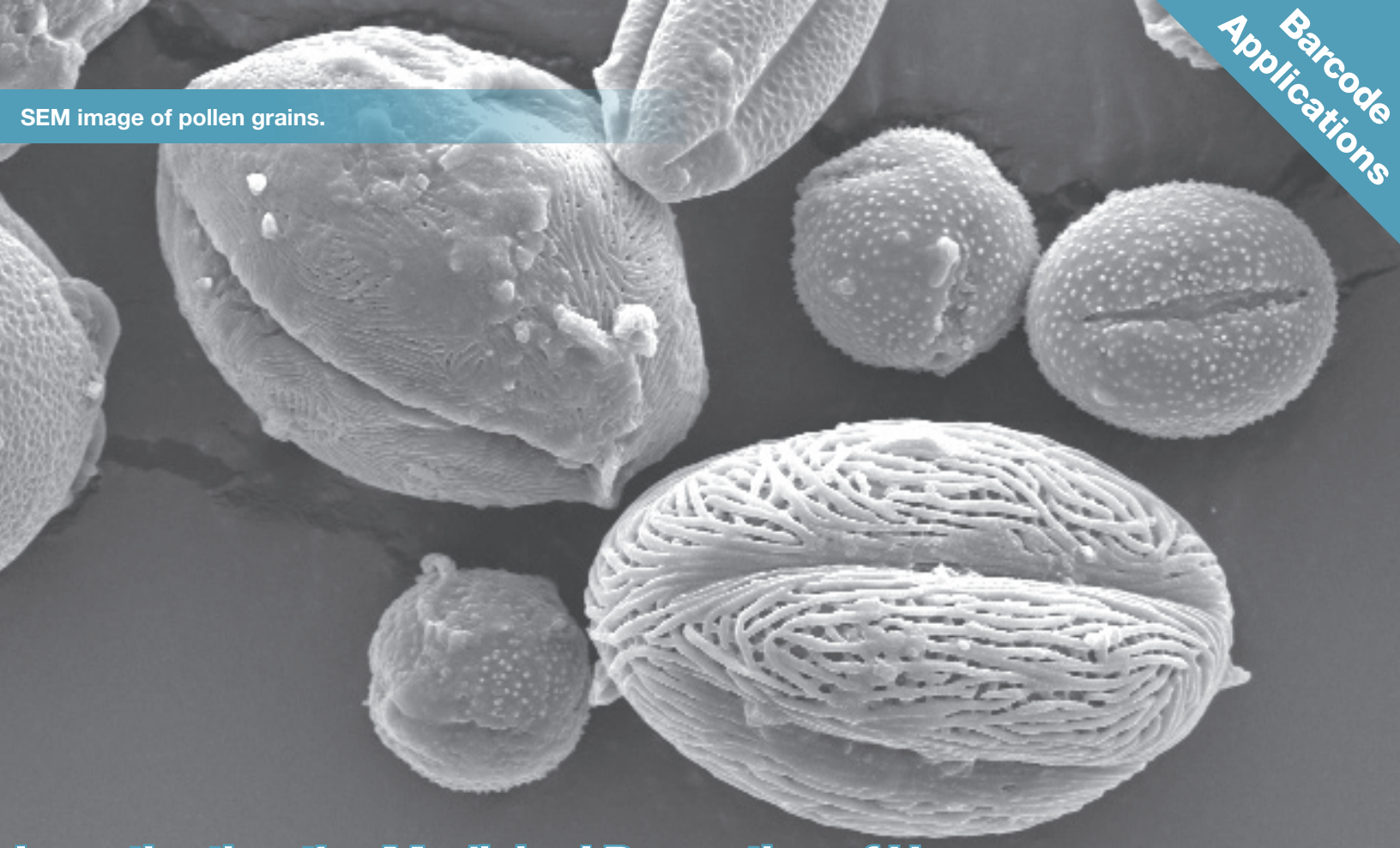
On behalf of the Conference Operating Committee (including Mehrdad Hajibabaei, Robert Hanner, and Paul Hebert), we would like to sincerely thank the members of the Local Support and Scientific Organizing Committees as well as thank all of the wonderful Conference Volunteers for contributing to the great success of this conference. Thank you also to the co-chairs and all participants in the important discussion on the formation of the International Society for the Barcode of Life. We also greatly appreciate the generous contributions of the conference sponsors. These sponsorships enabled the travel awards program, student and postdoctoral prizes, special plenary session, as well as key social and culinary events.

Finally, thank you also to all delegates for making the journey to Guelph and for sharing your work and ideas. It was our great pleasure to host you. We hope you left Guelph inspired and that you made new connections to foster the next stages of your work. We look forward to seeing you again in 2017 for the 7th Conference!

Conference delegates attending the Gala Reception at Cambridge Mill.

Above: Plenary presentation by Charles Godfray.

Below: Final farewells from Paul Hebert following his closing remarks at War Memorial Hall.



SEM image of pollen grains.

Investigating the Medicinal Properties of Honey

Written by and images by: Jennifer Hawkins (National Botanic Garden of Wales and Cardiff University, Wales) and Natasha de Vere (National Botanic Garden of Wales, Wales)

Understanding the floral composition of honey has a wide variety of applications. The traditional approach involves the microscopic examination of pollen present in honey, but this approach is time-consuming and requires specialist knowledge and expertise. DNA metabarcoding has the potential to revolutionise pollen characterisation as no prior knowledge of the plant species is required. DNA metabarcoding can be used to determine honeybee foraging preferences and to investigate the authenticity of honey, along with its botanical and geographical origin. Characterising pollen extracted from honey also allows the identification of the plant species that may be contributing to the antimicrobial properties of a sample.

A range of factors contribute to the therapeutic properties of honey, including high sugar content, low pH, hydrogen peroxide, and bee-derived peptides. Honey also contains antimicrobial phytochemicals which are yet to be fully characterised, representing a rich source of leads for the development of drugs for the treatment of microbial infections.

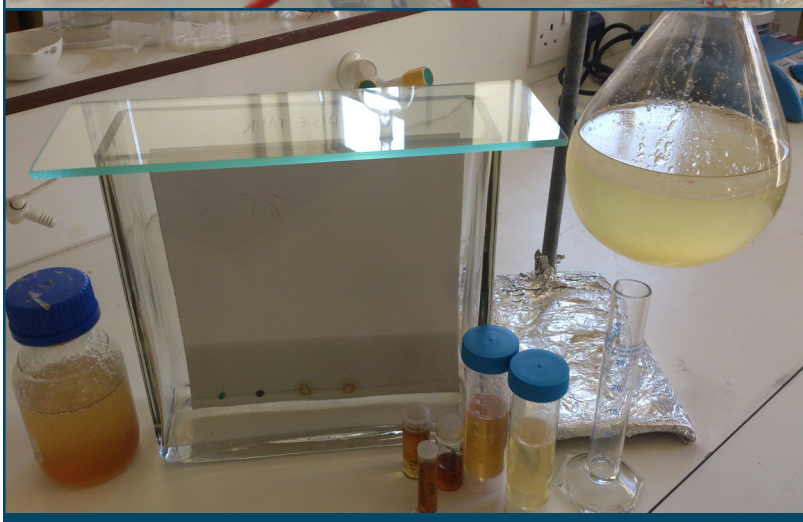
Over 200 honey samples donated by UK beekeepers were screened at Cardiff University for the presence of novel antibacterial compounds. Optimised agar well diffusion and broth microdilution assays were used to investigate the antibacterial activity against the healthcare-associated infection methicillin resistant *Staphylococcus aureus* (MRSA). In total, 88% of the honeys tested showed inhibitory activity; two of these samples were found to contain potentially novel antibacterial compounds, which were likely to be plant-derived.

A novel DNA barcoding application which could provide leads to new therapeutic compounds.

Pollen was extracted and characterised from twenty honey samples utilising the plant barcoding maker *rbcL*. Microscopic analysis was carried out on nine of the samples for comparison. DNA metabarcoding (454 and Illumina) was performed, with particular attention paid to the two samples which showed high levels of novel activity in the preliminary antimicrobial screen. Accurate species identification using DNA barcoding requires comprehensive databases of known reference samples. The Barcode Wales project created a rich reference database of DNA barcodes for the Welsh native flora. Plant profiles were produced using the three techniques to portray the composition of the honeys.

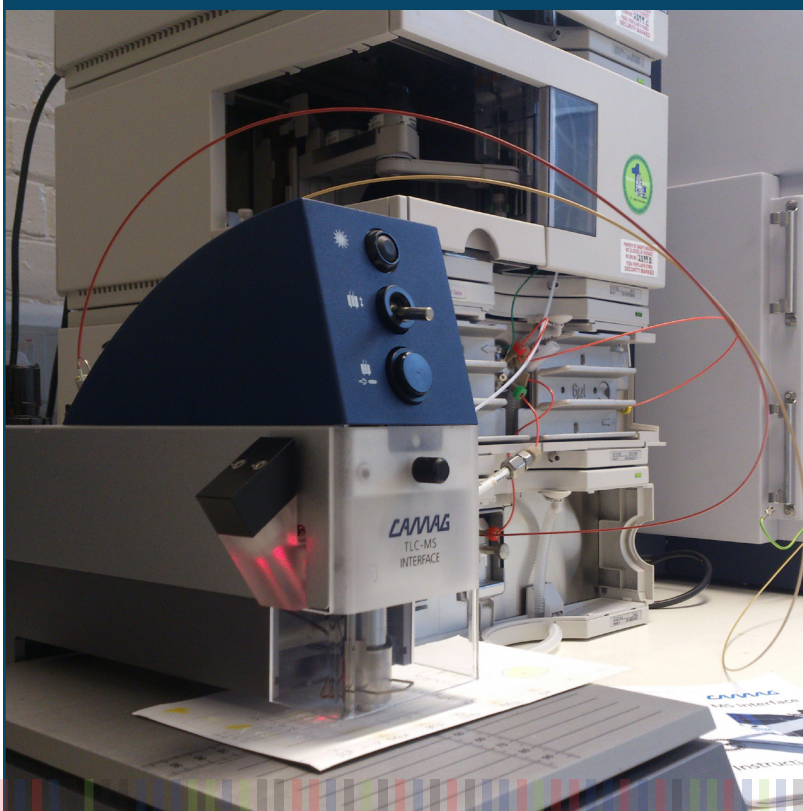
DNA metabarcoding provided superior discrimination for some plant families and greater repeatability compared to microscopic analysis. Interesting species identified in the two antibacterial samples included woodruff (*Galium odoratum*), bluebell (*Hyacinthoides non-scripta*) and dandelion (*Taraxacum officinale*). Organic solvent extracts from active honeys and characterised plants demonstrated antibacterial activity against MRSA, *E. coli* and *P. aeruginosa*. Activity-guided characterisation using a thin layer chromatography/mass spectrometry interface and high performance liquid chromatography was performed. Compounds identified using these approaches included known pinobanksin derivatives and unknown compounds suggesting that the plants may be the original source of active compounds. By combining antimicrobial screening, DNA metabarcoding and analytical chemistry, this novel application may provide new lead compounds that could serve as selective agents against many antibiotic resistant bacteria.

This study was part of the PhD project "Investigating Antibacterial Plant-Derived Compounds from Natural Honey" funded by the Knowledge Economy Skills Scholarships (KESS). For more information about the methods and results discussed in this article, see DOIs: 10.1371/journal.pone.0134735, 10.1371/journal.pone.0037945



Above: The chemical analysis of honey extracts using TLC.

Below: The TLC/MS interface used for activity-guided compound characterisation.



Kazuya Naoki and Isabel Gómez obtaining an e-voucher during one of several field trips in Bolivia.

Continental-scale Assessment of Avian Diversification

Written by: Pablo D. Lavinia (Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" – CONICET, Argentina)

In 2005, the Museo Argentino de Ciencias Naturales (MACN) joined the All Birds Barcoding Initiative aiming at generating DNA barcodes for the Argentinian avifauna. Three years later, and as part of Argentina's role as a regional node in promoting barcoding activities in neighbouring countries, the MACN started to perform field trips to Bolivia in collaboration with researchers from the Universidad Mayor de San Andrés, the Colección Boliviana de Fauna and the Museo de Historia Natural Noel Kempff Mercado. This expanded the geographic limits of our barcoding project and led to the creation of a DNA barcode library for the birds of Argentina and Bolivia, which currently holds almost 4,000 COI sequences from over 730 species and is still growing.

This library not only allowed us to test the efficiency of this tool in discriminating avian species of the region but also to explore avian diversity in the Southern Cone of South America. This screening revealed a large set of diverse and complex geographic patterns of deep intraspecific differentiation in the COI gene. One of the cases that stood out was the Red-crowned Ant Tanager (*Habia rubica*), for which we found 7% divergence between allopatric populations from the Atlantic Forest in Argentina and the Yungas-Amazonia complex in Bolivia.

Independently, at the Fourth International Barcode of Life Conference in 2011 in Adelaide, Australia, Patricia Escalante from the Universidad Nacional Autónoma de México presented the results of the project to barcode the birds of Mexico and Guatemala and showed a similar pattern of east-west divergence between disjunct populations of *H. rubica* in Mexico and Middle America. This was the keystone to a multi-institutional collaboration and allowed us to achieve the comprehensive geographic sampling needed to unveil the evolutionary history of *H. rubica* at a continental scale. Lastly, by the end of 2013, researchers from Colombia and Brazil, who previously worked with avian DNA barcodes, joined the project and the team was fully assembled.

This joint effort of Argentinian, Bolivian, Brazilian, Colombian and Mexican researchers resulted in a paper recently published in *Molecular Phylogenetics and Evolution*. For this study we sampled 100 individuals of *H. rubica* from Mexico to Argentina covering its entire distributional range and performed phylogenetic, phylogeographic and genetic population analyses based on COI and three other mitochondrial and nuclear genes. We complemented the genetic evidence with the assessments of coloration and behavioural differentiation.

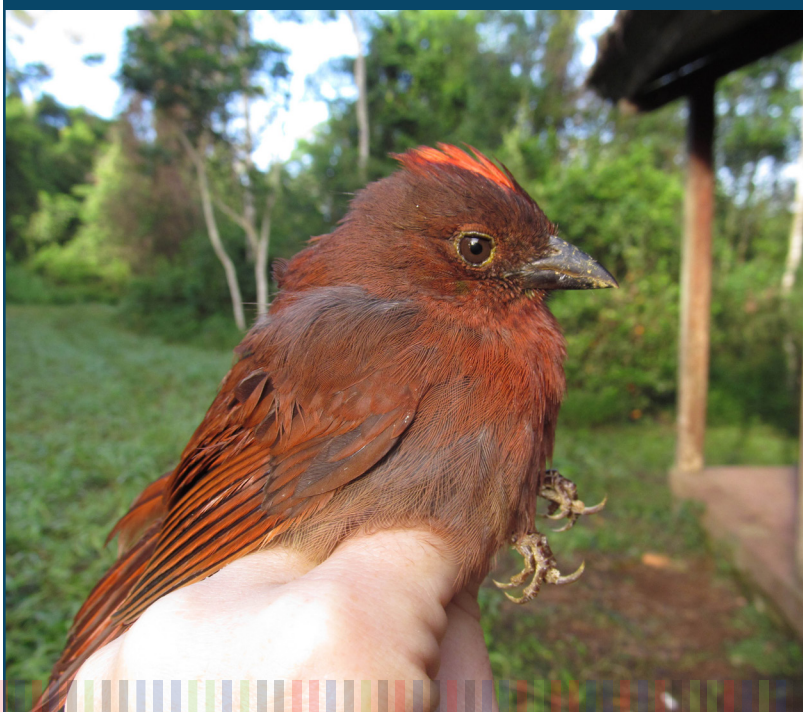
Local screening of diversity through DNA barcodes can grow into large-scale, multi-institutional collaborative projects.

Our results suggest that the evolutionary history of *H. rubica* through the last 5 million years has been shaped by the uplift of the Northern Andes, the formation of the Isthmus of Panama, the establishment of the open vegetation corridor that currently separates the Amazon and Atlantic forests, and Quaternary climatic fluctuations. This resulted in levels of genetic, morphological and behavioural divergence that justify considering at least three different species within what is currently known as *H. rubica*.

We believe that, aside from our specific findings on the diversification history of *H. rubica*, this particular study clearly illustrates how local screening of diversity through DNA barcodes can grow into large-scale, multi-institutional collaborative projects able to provide meaningful insights into the evolutionary history of certain regions and taxa of interest. This emphasizes the collaborative atmosphere and spirit that characterizes the DNA barcoding community. Examples like this should encourage researchers worldwide to cooperate in order to reach greater and more ambitious goals.

For more information on the results discussed in this article, see DOIs: 10.1371/journal.pone.0004379, 10.1111/j.1755-0998.2008.02218.x, 10.1371/journal.pone.0028543, 10.1016/j.ympev.2015.04.018

Males of *Habia rubica* from the Yungas and Amazonia complex in Bolivia (above) and the Atlantic Forest in Argentina (below).



Barcoding Reveals High Diversity in the Head Louse

Written by and image by: Muhammad Ashfaq (Biodiversity Institute of Ontario, Canada)

The relationship between the head louse, *Pediculus humanus*, and humans has been longstanding. This obligate human parasite is not only a pest and disease vector, its coevolution with *Homo sapiens* has been the subject of studies on anthropology and human evolution. Both mitochondrial and nuclear genes have been employed to analyze genetic diversity in *P. humanus* indicating that the head louse includes three mitochondrial clades (A, B, C) with differing geographic distribution; clade A is distributed globally, clade B is found in Australia, Europe and the New World, while clade C is only known from Africa.

In a recently published study in the journal Scientific Reports (see DOI: dx.doi.org/10.1038/srep14188), researchers from the Biodiversity Institute of Ontario, in collaboration with colleagues from Pakistan, determined that *P. humanus* is a complex of five clades. In addition to the three established clades (A, B, C), the study discovered two new clades, D and E, present in South Asia and Africa, respectively. The study also detected the presence of clade B in Africa, which was previously thought to be limited to Australia, Europe and the New World.

The analysis was based on 960 COI (barcode) and 479 cytb sequences from head lice from 30 countries with 693 barcode and 239 cytb sequences generated for the study. The data were analyzed by three species delineation methods (Barcode Index Numbers, Poisson Tree Processes, and Automatic Barcode Gap Discovery) to ascertain the number of OTUs and two phylogenetic methods (Neighbor-Joining and Bayesian Evolutionary Analysis) to determine clade support.

The study suggests that the earliest split of *P. humanus* clades occurred slightly more than one million years ago (MYa) and the latest about 0.3 MYa. The high sequence divergence in COI and cytb between the five clades of *P. humanus* is likely the result of both rate acceleration and the acquisition of lice clades from several archaic hominid lineages.

Cynopterus **bat caught during a night of mist-netting at an urban park in Kuala Lumpur as part of the fieldwork conducted under this program.**

Image credit: Lim Aik Hean

Research Network Launched

Written by: John-James Wilson (University of Malaya, Malaysia) and Elizabeth L. Clare (Queen Mary University of London, UK)

Species interactions hold ecosystems together. Such interactions are the building blocks of interaction networks (food webs) and their analysis allows us to predict ecosystem responses to environmental change. Urbanisation of a landscape is a significant challenge to the maintenance of ecosystem functions, disrupting the distribution of native species and potentially encouraging range expansion of non-native species.

The benefits of DNA barcoding to help resolve and categorise species interactions were quickly appreciated (for example in diet analysis – see Barcode Bulletin 1(2): 8-9, and ecological network analyses – see Barcode Bulletin 4(2): 13) and this area has become a focus within the international DNA barcoding community (Adamowicz 2015, DOI: 10.1139/gen-2015-0094). A special parallel session, chaired by ourselves, was devoted to "Trophic interactions of Mammals" at the 6th International Barcode of Life Conference.

We recently received funding from the Newton-Ungku Omar Fund to establish a research network investigating animal-plant interactions in urban environments in Malaysia. The Newton Fund is part of the UK Government's official development assistance program and is managed by the British Council with the goal of promoting the economic development and welfare of poor people in partner countries through science and innovation partnerships. Our network is particularly focused on student-driven collaboration.

Research network investigates animal-plant interactions in urban environments.

Newton-Ungku Omar Fund

Our inaugural network meeting took place at the Biodiversity Institute of Ontario as a pre-conference workshop under the 6th International Barcode of Life Conference. The core team from Queen Mary University of London, UK (QMUL) and the University of Malaya, Malaysia (UM) were joined by participants from Canada, Mexico, New Zealand and Spain.

"...during the workshop we heard about projects involving butterflies, bees, blowflies, Malaise trap insects, leeches, mussels, squids and fishes."

Our network has a particular interest in the interactions of bats, but during the workshop we heard about projects involving butterflies, bees, blowflies, Malaise trap insects, leeches, mussels, squids and fishes. The workshop included discussions on DNA barcoding – terminology, challenges and misconceptions, particularly with regards to the move to high-throughput sequencing data (by John-James Wilson), an overview of diet and food web analyses using DNA barcoding (by Elizabeth Clare), a background on food web and network analyses (by Tim Bartley, University of Guelph), and an introduction to the urban bats of Kuala Lumpur (by Voon-Ching Lim, UM).

Pei-Yin Ng from UM said: "I had the opportunity to meet the experts from different fields from all over the world and, most importantly, this workshop gave me a clearer picture on the possibility of DNA barcoding as well as the depth and width of research which can be conducted with this tool."

The highly successful inaugural meeting will be followed by student and faculty exchange between QMUL and UM early next year.

Introduction to Malaysian bat studies by David Bennett (Queen Mary University of London).

Monopis laevigella (Tineidae)

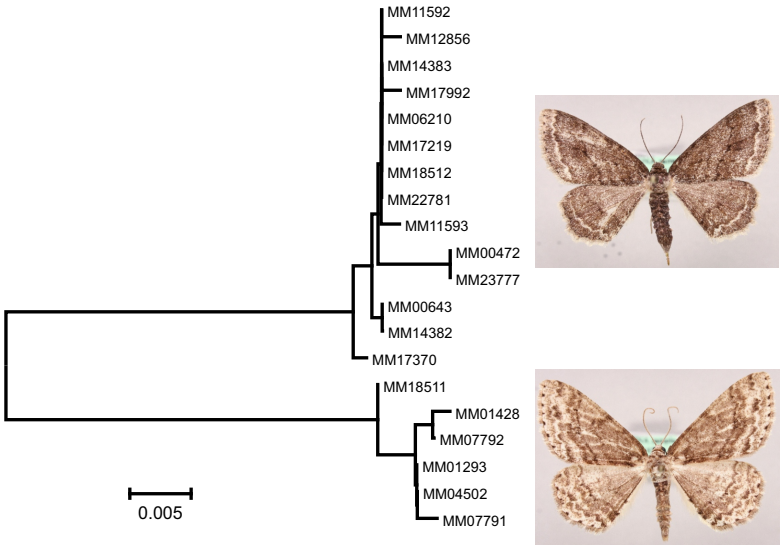
What is Behind Deep Intraspecific Barcode Splits?

Written by and images by: Marko Mutanen (University of Oulu, Finland)

While there is now a massive body of evidence indicating that animal DNA barcodes are efficient in telling apart species of various animal taxa, some studies have reported failures in certain cases. These failures are typically due to the incapability of DNA barcodes to differentiate between two, seldom more, closely related species. In other cases the utility of DNA barcodes is compromised by, at first sight, a very different phenomenon, namely extraordinary variability within a species. This may involve assignment of barcodes of a single species in two or more BINs, or even different barcodes becoming interspersed among species.

But why do barcodes show immense variability in some species? This question was asked by M.Sc. student Jonna Hänninen in a study conducted at the University of Oulu, Finland. As part of the Finnish Barcode of Life project, a library was built for 2,540 of ca. 2,600 species of butterflies and moths reported from Finland. Barcoding of 10,843 specimens revealed that 6.22% of species show over 2% intraspecific variability in their barcodes; most of these represent cases with two distinct groups of barcodes without intermediates found within a species. As many species are represented by only a single individual, the actual incidence of deep splits is likely higher. Deep intraspecific barcode splits are thus frequent, but so far little is understood about the causes.

Example of a deep intraspecific split. An externally variable looper moth *Ectropis crepuscularia* shows extraordinary, about 7%, intraspecific variability in DNA barcodes. The variation is however not continuous, but highly clustered forming two widely separate groups. From morphological grounds, it has been long debated whether this species is actually two closely related species. No evidence was found to support that in nuclear DNA markers but one of the groups is infected by *Wolbachia* while the other is free of it.

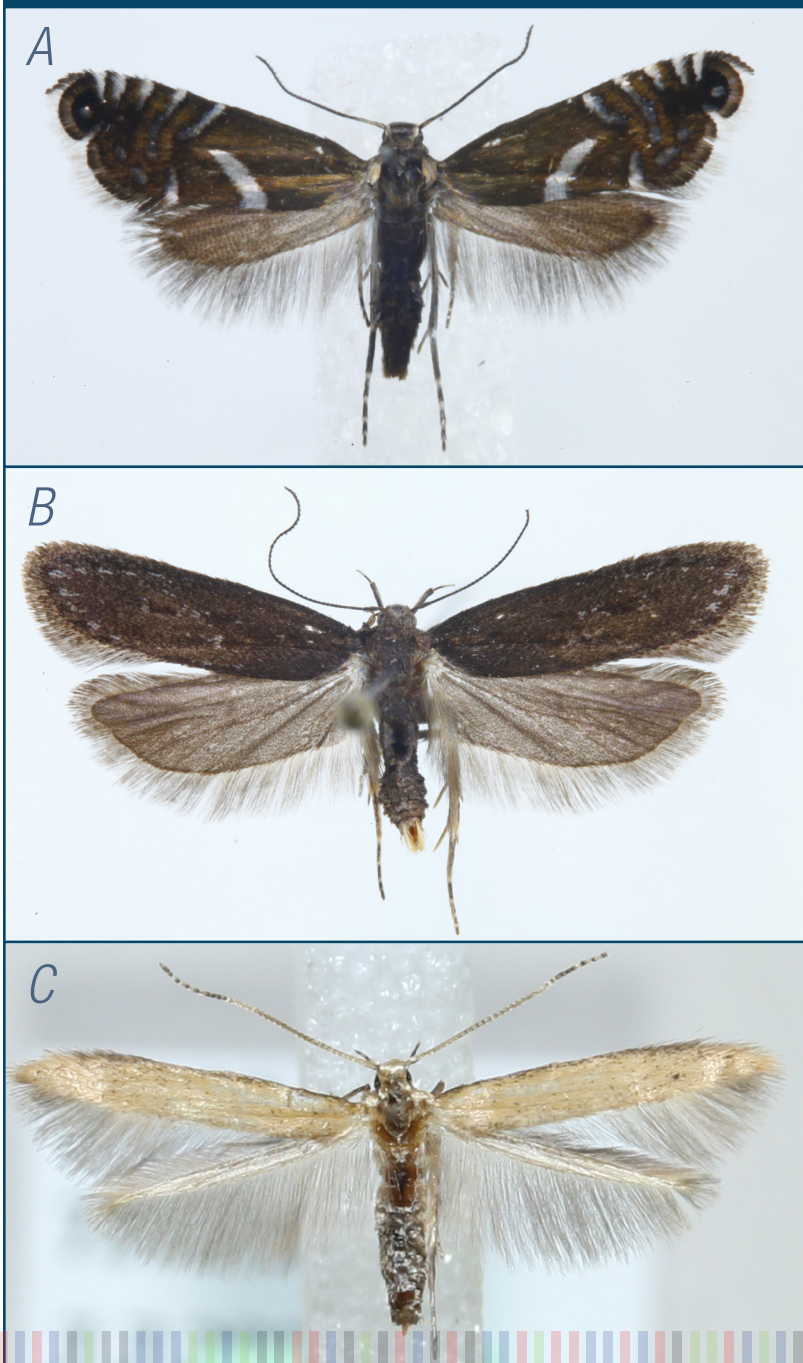


From the very beginning of DNA barcoding, it has been recognized that some of these splits actually indicate the presence of cryptic species. Indeed, many new species originally detected by barcodes have now been described. But this is not all. Deep splits may also be the result of retained ancient polymorphisms, similar to the polymorphism found in the human blood antigens of types A and B. Perhaps more importantly, such polymorphisms may be the result of mitochondrial introgression (a shift of the mitochondrial genome from one species to another). If this shift is accompanied by a new type (strain) of endosymbiotic *Wolbachia* bacteria, the effects may be wide-ranging. The presence of the bacteria may cause a rapid selective sweep and the occurrence of a new type of barcode in a species resulting in temporarily shared barcodes between two species until they have again evolved in different directions as well as the co-presence of very different barcodes in a population, both associated with different *Wolbachia* strains.

All of these hypotheses lead to different predictions. If cryptic species are involved, one should expect the split to be present in nuclear genes, while the ancient mitochondrial polymorphism and *Wolbachia* hypotheses predict the opposite. Provided *Wolbachia* is behind it all, one should expect different barcode clusters being linked with different *Wolbachia* strains, or one strain with and another without *Wolbachia*. Luckily, detecting *Wolbachia* is relatively straightforward and many suitable markers of nuclear DNA are available for butterflies and moths.

Detailed study of 29 species provided evidence that at least in four, but possibly in as many as eight species, the split is due to cryptic diversity. This is astonishing because the Finnish butterfly and moth fauna is taxonomically among the best investigated in the world. But the real surprise was that in as many as 22 cases out of 29, the barcode clusters were associated with different *Wolbachia* strains, though this count includes species that showed cryptic diversity (different species may naturally host different *Wolbachia* strains). While more poorly investigated regions will likely show a higher proportion of cryptic species among deep splits, it seems evident that *Wolbachia* is another major cause behind intraspecific barcode splits, which should be taken into consideration in taxonomic studies utilizing DNA barcode libraries.

In addition to *Monopis laevigella* (pictured at the top of the previous page), these three species have been shown to include a cryptic, sympatric, and morphologically indistinguishable sister species. In each of these cases, the split observed in the DNA barcodes was repeated in several nuclear markers, providing strong evidence for reproductive isolation. A. *Glyphipterix forsterella* (Glyphipterigidae), B. *Neofaculta infernella* (Gelechiidae), C. *Batrachedra pinicolella* (Batrachedridae).



Range of plant parts for sale at Faraday Market.

Survey of Traditional Medicinal Bulbs Traded at a Street Market

Written by: Dorcas M. Lekganyane, Herman van der Bank, and Michelle van der Bank (African Centre for DNA Barcoding, University of Johannesburg, South Africa)

The term "muthi" is often associated with some kind of witchcraft or sorcery, making a visit to a traditional healer taboo. However, approximately 80% of South Africans still use traditional medicine ("muthi") as an important component of primary health care. Traditional medicine is not considered inferior but is viewed as an alternative to western medicine. Thus commercialization of this trade has led to an increase in the number of Endangered or Near Threatened species due to overharvesting. Importantly, 86% of harvests result in the death of the plant, resulting in over-exploitation and noticeable levels of species depletion. Specialized gatherers harvest these plants mostly from wild resources, which reach the market via a middleman.

"...commercialization of this trade has led to an increase in the number of Endangered or Near Threatened species..."

Faraday is the second-largest traditional medicinal market in the country with >400 traders and humming with people pretty much any time of day searching for herbs, animal skins and derivatives, and healing potions. Whole plants, roots and bulbs account for approximately 50% of the 500 species and 48% of the volume of plants sold at Faraday. Here, plants are traded using local names, which do not correspond to scientific names. Furthermore, adulteration of the plant and morphological similarities or the lack thereof, makes identifying samples at a taxonomic level challenging.

In this study we measured the efficiency of DNA barcoding in identifying medicinal bulbous plants traded at Faraday. Plants were collected at the beginning of every month. Traders were questioned about the medicinal uses of each plant along with their vernacular names. Over 90 bulb samples were collected and, with each monthly visit, several new samples were recorded. Standard DNA barcoding protocols were used to sequence the core barcoding regions. Vernacular names were used to match scientific names of each plant using a checklist by Williams (2003) to generate an identification table. Notably, Williams (2003) succeeded in identifying 68 plants over a three year period compared to 61 species identified in less than seven months in this study.

PCR amplification produced 100% success; however sequencing success was higher for *rbcLa* (91%) than *matK* (78%). BLAST searches using *rbcLa* resulted in high numbers of ambiguous identifications whereas searches using *matK* were less ambiguous, allowing the majority of samples (98%) to be identified to genus level. When using a tiered approach (*rbcLa* + *matK*), most samples could be identified to a species level. Only 36% of the Williams (2003) samples could be identified to a species level whereas, with DNA barcoding, 90% of samples were identified to a species level providing a 54% increase in identification efficacy when applying DNA barcoding as opposed to traditional taxonomic methods.

Compared to traditional taxonomic methods, DNA barcoding provided a 54% increase in identification efficacy.

Our survey indicates a slight increase in the number of species traded at the market since 2003. Red list status confirmed the majority of species (>75%) as currently not endangered whilst 13% of species traded are declining or Near Threatened in the wild. The figure on the right shows some Near Threatened bulbs that were identified; for example, it is estimated that around 38,000 bulbs of *Merwilla plumbea* are sold annually at Faraday.

In conclusion, the findings of this study have major applications for the monitoring of trade in endangered species at traditional medicinal markets in South Africa. Given the short time frame of this study, the mere presence of any Vulnerable or Near Threatened species is astonishing and poses a serious conservation issue, assuming that if trade continues in such an unsustainable manner, these plants could swiftly become Critically Endangered in the near future.

Above: Dorcas Lekganyane collecting bulb specimens at Faraday.

Below: Two Near Threatened bulbs identified at the Faraday Market (http://redlist.sanbi.org), *Eucomis bicolor* (Baker) on the left and *Merwilla plumbea* (Lindl.) Speta on the right.

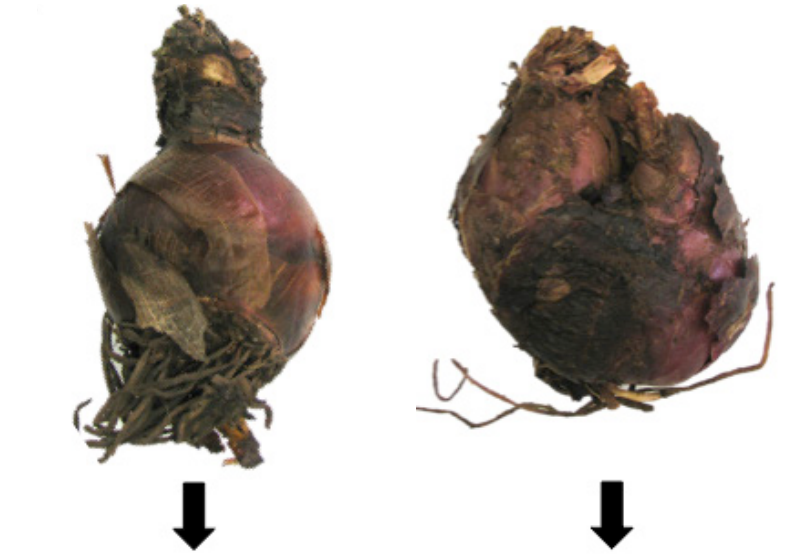


Image credit: Clare Beet

Barcoding Antarctic Springtails

Written by: Clare Beet and Ian Hogg (University of Waikato, New Zealand)

The Antarctic terrestrial environment is one of the harshest on earth. Repeated glacial cycles over the past 80 million years have created a landscape characterised by low biodiversity. However, it hasn't always been this way with many of the current inhabitants persisting as remnants from much warmer times when dinosaurs once roamed the continent. Now, the largest year-round terrestrial animals on the Antarctic continent are the considerably smaller, but equally charismatic, Collembola or springtails at less than 1.4 mm body length.

Springtails were first observed by members of Captain Robert F. Scott's expeditions to the Ross Sea region in the early 1900's. The region encompasses almost 30 degrees of latitude and includes one of the most prominent geological features, the Transantarctic Mountains, as well as much of the biological diversity. It is also home to the Western Antarctic Ice Sheet (WAIS), one of the largest ice sheets on the planet. Even here, the landscape is 98% covered with ice and, for those few exposed areas, the 2% is very dry with most life existing where there is at least some access to water such as near glaciers, meltwater streams or even small snow patches.

Biological survey work in the early 1960's expanded the earlier observations from Scott's time to reveal three biogeographic zones each with three, and in one case four, unique species for a total of 10 springtail species within the Ross Sea region. However, much of the earlier work was based on classical taxonomic assessments and had been undertaken primarily in the vicinity of existing bases or camps – the wider region was still vastly underexplored.

"...barcoding was to reveal considerably more diversity and knowledge of the Antarctic landscape than we initially thought."

Since the late 1990's, Antarctica New Zealand, and more recently the New Zealand Antarctic Research Institute, have been supporting efforts to better understand patterns of biodiversity within the Ross Sea region. This has enabled visits to some of the more remote regions and has allowed coverage as far south as 87° – the southern-most exposed rock in Antarctica. The University of Waikato has been actively involved in this work for over 20 years and in particular the use of molecular techniques such as DNA barcoding. Paul Hebert was a team member on one of our earlier (2001) field expeditions and suggested that we trial his idea for "DNA barcoding" on the springtails. Given that there were so few species, he even quipped that "we could finish the task in the morning, and move on to something more interesting in the afternoon". However, barcoding was to reveal considerably more diversity and knowledge of the Antarctic landscape than we initially thought.

DNA barcoding of samples from the southern-most biogeographic zone (>79° S) revealed at least three "new" species. In the northern-most zone (<75° S), detailed coverage of a single species by Italian and Australian colleagues revealed four unique Barcode Index Numbers (BINs), strongly suggesting the presence of cryptic species. Our most recent work this past season in the in-between or "middle" zone has revealed a total of seven BINs from the original three species.

Using molecular clock dating techniques, the divergences among several of these BINs have corresponded to roughly 3-5 million years ago – precisely the time the WAIS was thought to have completely collapsed. This resulted in sea levels rising and increased dispersal opportunities for Collembola via meltwater streams and open seaways before the WAIS eventually reformed. The various BINs appear to have remained in relative isolation ever since. All records are available on the Barcode of Life Data Systems (BOLD).

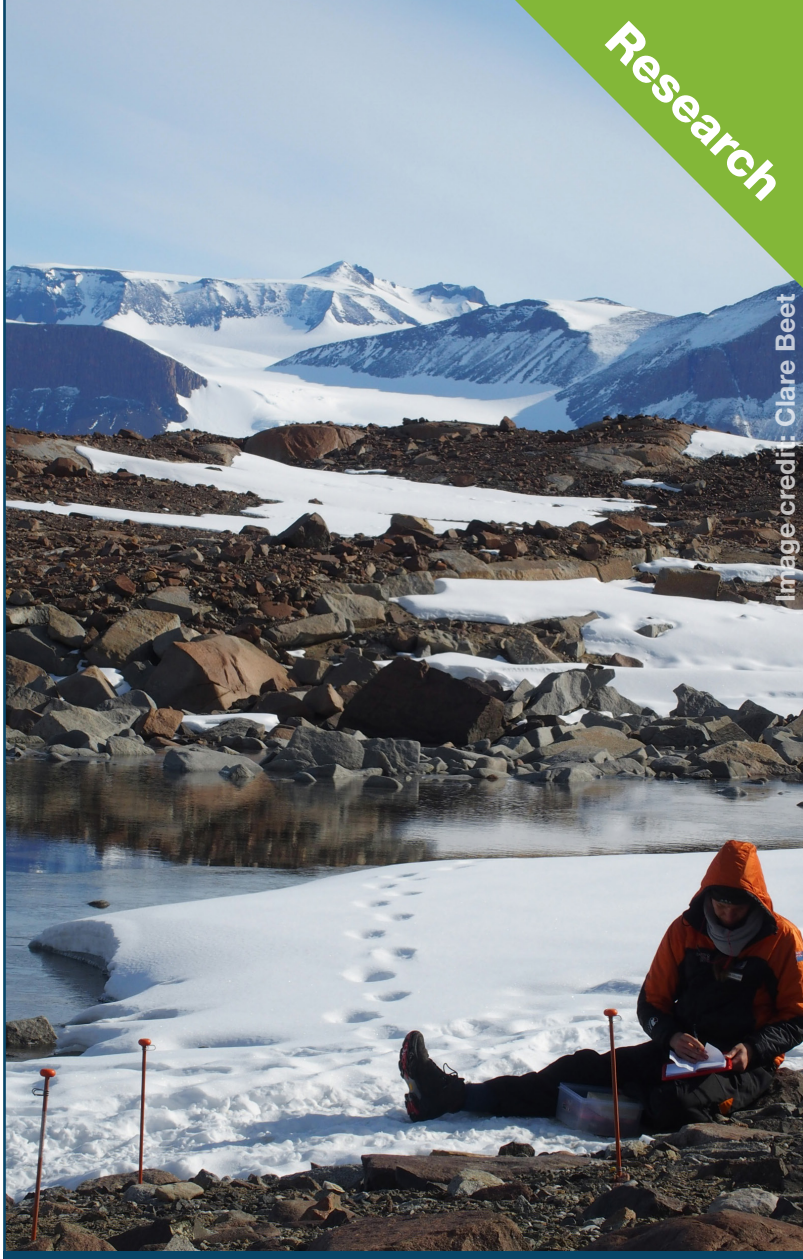


Image credit: Clare Beet

Above: Gemma Collins collecting springtails in pitfall traps near a meltwater pond on Mt Seuss.

Below: Springtails floating in a rock pool above Benson Glacier.

Image credit: Gemma Collins

Conference Reflections from a Non-scientist

Written by: Joanne Pearce (University of Guelph, Canada)

Attending an international DNA barcoding conference is an opportunity that does not come by very often — especially for someone with no background in DNA barcoding.

This past summer however, the Biodiversity Institute of Ontario (BIO) hosted a slew of international researchers for the 6th International Barcode of Life Conference — which I was invited to attend as a SPARK journalist, covering the conference over Twitter. SPARK (Students Promoting Awareness of Research Knowledge) is the University of Guelph's Office of Research communication program that has student writers help make science understandable for the public. Being a journalist, it's not unusual to need to cover topics that I am not intimately familiar with.

"The sheer breadth of research explored at the conference was astounding."

I also had the opportunity to see the informational handouts that I had produced over the summer for the conference being distributed, such as a piece called "10 things to know about DNA barcoding," which was created to aid the general public in understanding DNA barcoding.

Now, to share my experience, here are five things I learned from the conference that helped make it unforgettable:

Seeing international collaborations form

Watching international experts come together to decipher and disseminate knowledge is incredible. During the parallel sessions, researchers shared their subject matter expertise, and then names would be given and information exchanged afterwards for discussions to continue on after the conference. The wide-ranging passion for DNA barcoding techniques and applications spoke so loud that, even without expertise in this area, I was thrilled to be a part of it all.

The diversity of content and sessions to attend

The sheer breadth of research explored at the conference was astounding. Having to choose which sessions to attend was difficult, as I knew I'd have to miss some. In the end though, it allowed me to choose sessions based on personal interests and gave me the chance to listen to the various areas where DNA barcoding is being employed. The range of the speakers' expertise, from PhD students to esteemed professors in their field, also contributed to the conference's inclusive atmosphere.

The River Run Centre's *State of Biodiversity* Special Plenary Session

What I loved about this session was that, as a public event, it was a night not just for scientists, but for the public too. One moment I was listening to the hilarious and insightful words of Laurence Packer on the state of bees, and the next I was hearing Bridget Stutchbury speak passionately about conservation of birds in Canada.

Networking and socializing

There is a difference between knowing about and experiencing a conference of this magnitude. Walking around the conference, it was easy to approach researchers from around the world and talk about DNA barcoding in their own field. I got to speak with researchers who compared opinions on topics covered at the conference and I learned about specific and emerging research areas such as eDNA.

Final Plenary: Reflections and looking forward

The closing remarks on the conference offered the chance to reflect on the exciting week and the future of DNA barcoding. With DNA barcoding's growing presence in research, the final plenary concluded what the conference had been discussing all along: DNA barcoding is a new frontier in understanding the natural world. As someone who strives to learn more about the world's processes, but doesn't necessarily have the academic knowledge, this is incredibly exciting to hear.

Barcoding the Swiss Lichens and Associated Fungi

Written by: Kristiina Mark (University of Tartu, Estonia) and Christoph Scheidegger (Swiss Federal Research Institute WSL, Switzerland)

Lichens are symbiotic organisms consisting of a fungal partner (also called the mycobiont) and one or more photosynthetic partners (the photobiont). Lichens are named for their fungal component. More than 20% of fungal species are lichen-forming, but they do not form a single monophyletic clade in the fungal tree of life, instead, multiple lichenization events have been suggested.

"... only a minority of lichen-forming fungi have been barcoded from the estimated 20,000 species."

While lichens play an important role in the ecological integrity of many vulnerable landscapes, only a minority of lichen-forming fungi have been barcoded from the estimated 20,000 species. For example, the largest and most typical order of lichen-forming fungi, Lecanorales, consists of about 5,700 species, but merely 29% of them have publically available ITS sequences (Internal Transcribed Spacer region, the official barcoding marker for fungi).

Barcoding lichens proves often quite challenging, especially when we work with small crustose lichens. In addition to sampling difficulties that arise when other very similar lichen species live close to or even intermingled with the target species, many saprophytic, endophytic, and parasitic fungi live intimately admixed with the lichen mycobiont, making the application of Sanger sequencing insufficient in many cases.

One hundred species, including macrolichens (foliose and fruticose thalli) and crustose lichens, were collected in Switzerland to test barcoding of the mycobiont using Roche/454 pyrosequencing, and additionally, study the diversity of lichen-associated fungi. We amplified the full ITS region of 100 different species using standard fungal specific primers and sequenced in the Roche GS FLX+ system using a typical amplicon sequencing approach. The sequencing produced almost 130,000 reads that, after data processing and clustering phases, resulted in 1171 fungal barcodes. Clustering of barcodes at 97% generated 567 operational taxonomic units (OTUs).

The majority of the barcodes from the targeted lichenized fungi were identified to the correct species (69) or at least to the correct genus (18) using the NCBI nucleotide database as reference. This indicates that GenBank, currently the most complete database for lichenized fungi, could be used as a reference database to identify common species. At the same time, one needs to be careful – 9 of our samples were identified to the correct genus but wrong species, and one sample to a completely wrong species with high identity score (≥97%). When using a tree-based identification approach, we found that misidentifications were mainly due to three reasons: (a) labelling or identification mistake; (b) incomplete reference database with missing species or partial sequences; (c) biological reasons, such as low genetic variation in young species complexes.

Additionally, we recovered unexpectedly high fungal diversity within the sequenced lichens, on average 10 fungal lineages per lichen sample. Two samples included the lichen mycobiont only. Altogether 469 lichen-associated fungal OTUs were barcoded, covering 22 different fungal classes. The fungal classes Dothideomycetes, Eurotiomycetes, and Tremellomycetes were most frequent and, within each of these classes, a single order was dominant, consisting of a high fungal diversity in the number of OTUs. These three fungal orders, Capnodiales, Chaetothyriales, and Tremellales, are also the taxa where the majority of lichenicolous fungi are found – fungi parasitic on lichens. At the same time, merely 15% of the lichen-associated fungal OTUs found in this study could be identified to species level, indicating that lichen-associated fungal diversity is greatly unexplored, and further studies on the functionality of these fungi and reasons for such high diversity are needed.

The study was funded by the Rectors' Conference of the Swiss Universities (CRUS) through the SCIEX-NMSch Scientific Exchange Programme, FOEN – the Federal Office for the Environment, and SwissBOL.

Image credits: Prof. Christoph Scheidegger, WSL

Above: *Fellhanera bouteillei* on *Abies alba* needles.

Below: Successful transplant of *Bunodophoron melanocarpum*.

Detecting and Deterring Fish Mislabelling in South Africa

Written by and images by: Donna-Mareè Cawthorn (Stellenbosch University, South Africa)

Seafood mislabelling has emerged as a globally pervasive problem, seemingly intensifying in synchrony with the ever-declining state of the world's fish stocks. Although driven primarily by economic gain, such practices are undoubtedly facilitated by the growth and globalisation of seafood trade, the increased processing of seafood into value-added commodities, as well as weak and/or poorly enforced regulations. Irrespective of the motives or complexities of control, the consequences of mislabelling are manifold and include financial, health and conservation impacts.

"Irrespective of the motives or complexities of control, the consequences of seafood mislabelling are manifold and include financial, health and conservation concerns."

DNA sequencing methods hold great promise for fish species authentication, given that DNA is present in all cells, the molecule is robust and the diversity afforded by the genetic code allows discrimination of even closely-related species. Nonetheless, in order to be useful in making accurate species identifications and tracking changes in seafood trading patterns over time, such methods must be backed up by robust reference DNA sequence databases and statistically sound sample collection protocols. This article describes our 8-year research effort in South Africa to systematically harness the power of DNA barcoding to detect and deter fish mislabelling in the country.

Following rigorous surveys of fish species occurrence on the South African market, multiple individuals belonging to 53 commonly available fish species were collected, expertly identified and vouchered. A comprehensive DNA sequence library was thereafter established from the reference samples based on the sequencing of three mitochondrial gene regions (cytochrome *c* oxidase subunit I [COI], 16S ribosomal RNA [rRNA] and 12S rRNA genes), followed by the submission of over 570 new reference sequences to GenBank and BOLD. COI barcoding was found to be capable of unequivocally identifying the species origin of the vast majority (98%) of fish examined, whereas discrimination of congeneric species was frequently problematic with 16S and 12S rRNA gene sequencing.

The next step was to utilize the established reference COI sequence library to conduct the first comprehensive evaluation of the prevalence of fish mislabelling in South Africa. In 2010, 248 samples were collected from seafood wholesalers and retail outlets in four South African provinces, namely the Western Cape, Eastern Cape, KwaZulu-Natal and Gauteng. Subsequent DNA barcoding revealed that 21% of collected fish samples were mislabelled in terms of species, including 31% from retailers and 9% from wholesalers.

"DNA barcoding has been confirmed as an extremely powerful method for the identification of fish species traded in South Africa..."

Owing largely to the considerable media attention generated by the aforementioned mislabelling study, a number of interventions have been implemented in South Africa since 2010 in an attempt to improve local seafood marketing transparency. These have included the promulgation of new food labelling regulations, increased authentication testing by industry, as well as the work of several NGOs to promote more comprehensive and standardised seafood labelling. In order to gauge the effectiveness of these initiatives, a follow-up market study was conducted in 2014, with DNA barcoding showing that 27 of 149 (18%) fish samples collected from South African restaurants and retailers were potentially mislabelled. While such improvements may not appear phenomenal, DNA barcoding has continued to enhance our understanding of both the regions where mislabelling remains problematic and the species most prone to deceitful trade, allowing key targets to be flagged for future resolution.

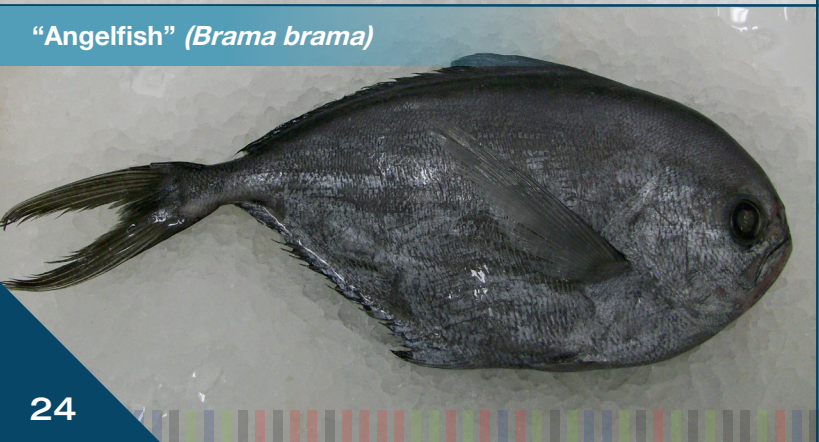
Overall, DNA barcoding has been confirmed as an extremely powerful method for the identification of fish species traded in South Africa, the utility of which has been strengthened by the generation of a reference DNA sequence library for commonly marketed species. Such an approach, when underpinned by structured and on-going seafood testing protocols, can provide an effective and economical tool for industry self-regulation, governmental monitoring, and prosecution of illegal activities.

For more information on the results discussed in this article, see DOIs: 10.1016/j.foodcont.2011.04.009, 10.1016/j.foodres.2011.11.011, 10.1111/j.1755-0998.2011.03039.x, 10.1016/j.gene.2011.09.009, 10.1016/j.foodchem.2015.03.113

Cape horse mackerel

Deep-water Cape hake

"Angelfish" *(Brama brama)*



Carpenter seabream

Kristy Deiner sampling the river Glatt in Switzerland.

Image credit: Mat Seymour

CSI for Biodiversity

Written by: Kristy Deiner (University of Notre Dame, United States)

For the past four years, I have been involved in several projects to test the utility of detecting species in their environment simply by finding the DNA they leave behind and identifying it using DNA barcoding. The use of DNA found in the environment (termed environmental DNA or eDNA) to detect species is at the forefront of new tools in the tool box for conservation and restoration and provides a non-lethal way to detect species. It can provide geographic information about the presence of species of concern and invasive species and it can even be used to conduct whole community assessments.

Environmental DNA provides an efficient and non-lethal way to detect species.

A clear demonstration across a broad array of species and freshwater habitats worldwide has culminated in a special issue released earlier this year by *Biological Conservation*, "Environmental DNA: A powerful new tool for biological conservation", edited by C. Golberg, K. Strickler and D. Pilliod.

In this special issue, my collaborators and I contributed a study that advances some of the fundamental and technical aspects for applying this tool in freshwater systems. Specifically, we tested different methods for concentrating DNA from water samples and purifying it for detection. Our results demonstrate that the use of particular methods, and specific combinations of those, can increase the likelihood of detecting species from trace sources of eDNA. These results give practitioners interested in applying this tool clear evidence of which methods can be successfully used depending on the species sought for detection.

In addition, our research has been focused on the detection of macroinvertebrates such as mussels and insects in rivers and lakes. Macroinvertebrates are an important group because they are used to monitor water quality all over the world. Biomonitoring with freshwater macroinvertebrates primarily works by relying on the detection of sensitive or tolerant families, genera or species in the environment. Then, based on the characteristics or phenotypes of the combination of species, data are interpreted as indicating the health or impairment of a river or lake.

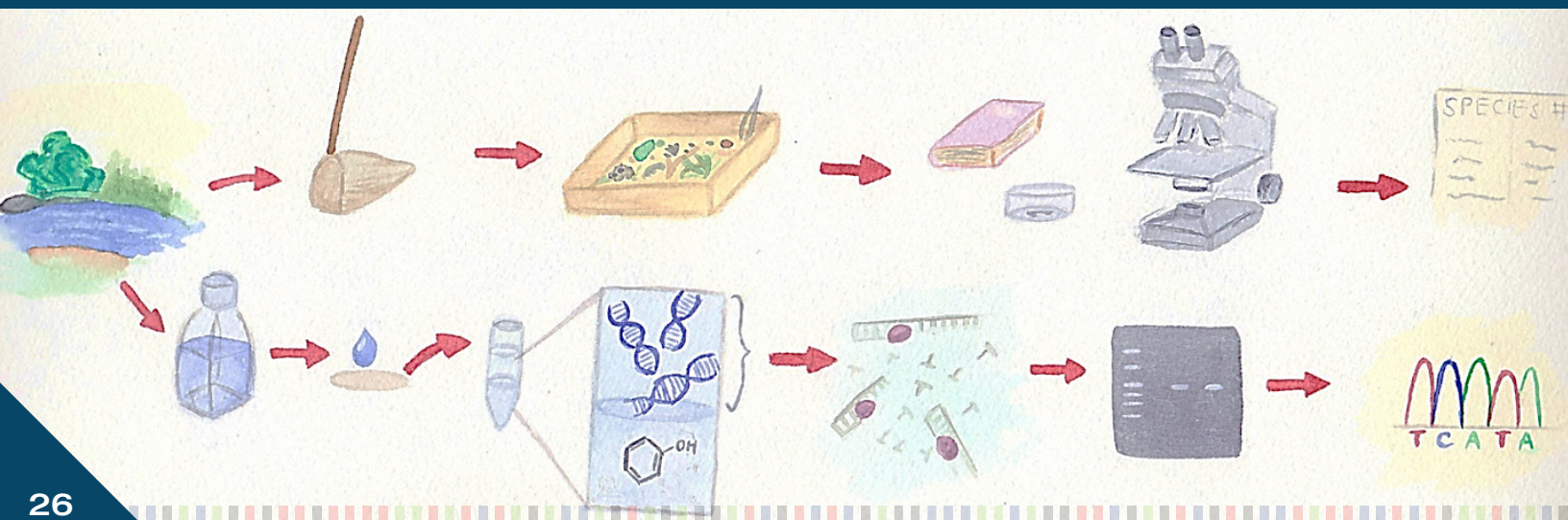
Macroinvertebrates are important worldwide as indicators of water quality.

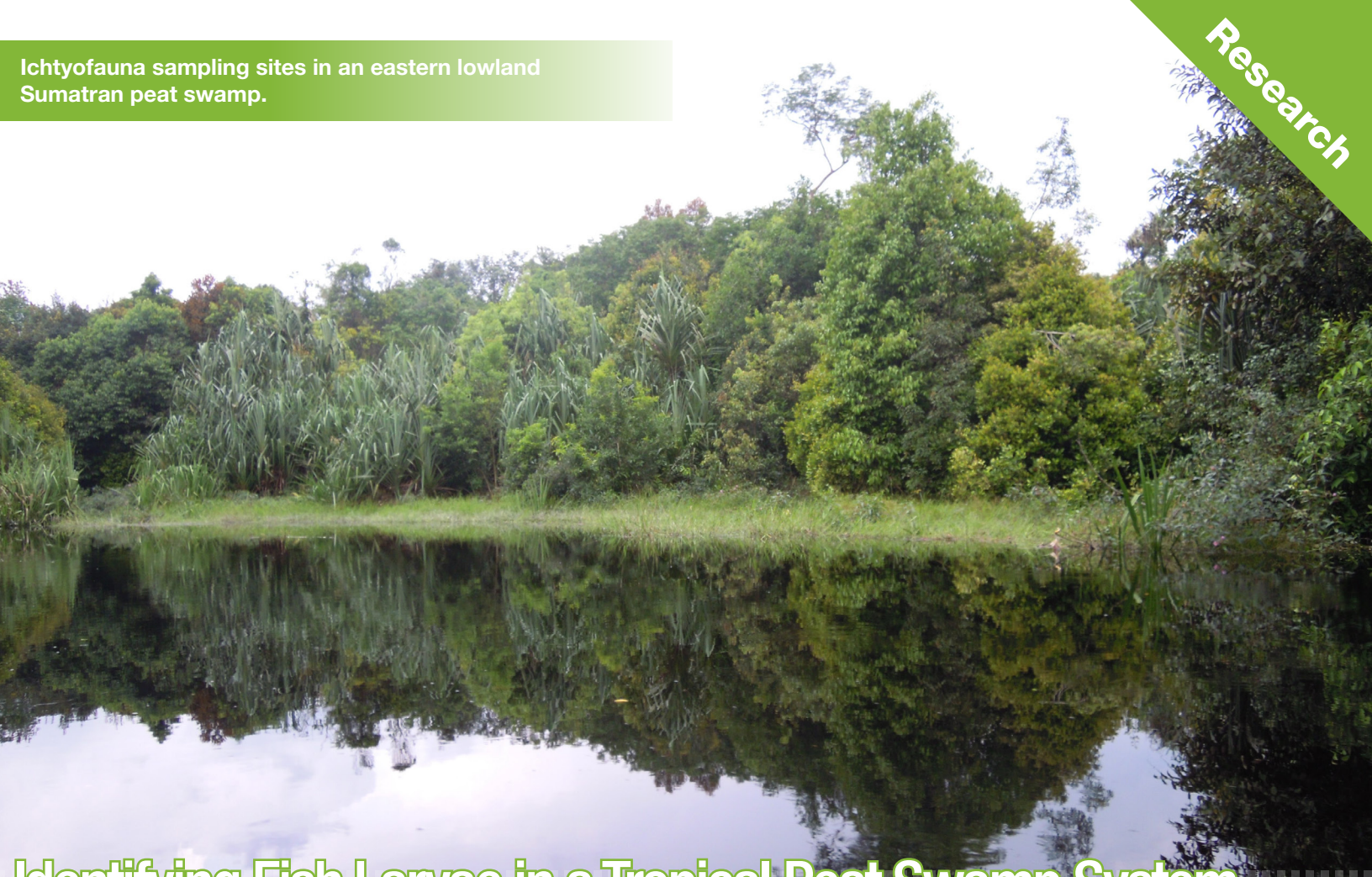
Conventional methods used to detect macroinvertebrate species rely heavily on lethal sampling methods and are very labor and time intensive due to the need for morphological identification of species. Our research team has demonstrated that we can use eDNA to detect macroinvertebrates spanning many different species and that DNA can be transported over large distances (10-12 km) in rivers. Our demonstration that this genomic tool provides an efficient and non-lethal way to do biomonitoring of freshwater systems has led to increased interest from management agencies all over the world.

The potential efficacy and power of using eDNA to detect and describe biodiversity is still being established and there remains room for substantial innovation and improvement. For example, we do know that eDNA methods used for species detection greatly increase the power of field surveys for rare and elusive species. There are, however, many inferential challenges to interpreting eDNA detections, including spatial and temporal considerations, relationships to abundance, and the potential for false positive and false negative detections. These challenges all need to be understood in order to move forward with producing robust and defensible eDNA studies for understanding the distribution of biodiversity. The ability to identify sequences found in the environment also relies heavily on having reference barcodes for comparison such that a name can be associated with each sequence we find. In the future, focusing on barcoding species that are important for biomonitoring is essential to make this tool valuable for policy makers and society.

For more information on the results discussed in this article, see DOIs:
10.1016/j.biocon.2014.11.018, 10.1086/678128, 10.1371/journal.pone.0088786, 10.1101/020800

Graphic of two sampling strategies: top row depicts the conventional process to sample macroinvertebrates from an environment and the bottom row depicts the environmental DNA process to sample the same diversity in a non-lethal way. Art by Elvira Mächler and used with permission.





Ichtyofauna sampling sites in an eastern lowland Sumatran peat swamp.

Identifying Fish Larvae in a Tropical Peat Swamp System

Written by and images by: Arif Wibowo (Research Institute for Inland Fisheries, Indonesia)

One of the most important natural ecosystems in the world is peat-land. It comprises a unique and complex ecosystem, which has a globally important role in biodiversity conservation at genetic, species and ecosystem levels and contains many species found only or mainly in peat-lands. These species are adapted to the special acidic, nutrient poor and water-logged conditions.

"Peat swamp ecosystems are considered one of the most threatened, neglected, and poorly understood biotopes..."

Peat swamp ecosystems are considered one of the most threatened, neglected, and poorly understood biotopes and their importance is underappreciated. The race to catalogue biodiversity before it disappears is particularly intense in the peat swamps. Most of fish biodiversity research in peat swamp systems relies on morphological diagnosis, primarily of adults and relatively large fish which can be morphologically distinguished. Despite the prevalence of planktonic larvae in tropical peat swamp systems, comprehensive larval identification keys for these systems are extremely limited making it almost impossible to identify larval specimens solely by their external appearance. In addition, in their early life, the morphology of a species can change quickly and significantly during its development from pre-flexion larvae to post-flexion to the pre-juvenile stage. Thus, the same species at different developmental stages may be identified differently when based on morphological characteristics.

A DNA-based identification system, founded on the mitochondrial gene cytochrome *c* oxidase subunit 1 (COI), can aid in the resolution of this diversity. This approach can be applied for diverse developmental stages, such as larvae of fishes and juveniles to discover diversity. Based on established DNA barcoding methodology, this study investigated known adult species within the peat swamp ecosystem as well as previously unknown fish species which are found only at larval stages within this unique ecosystem.

"Another interesting finding is the confirmation of a minimum of eleven species that spend their entire life in this ecosystem..."

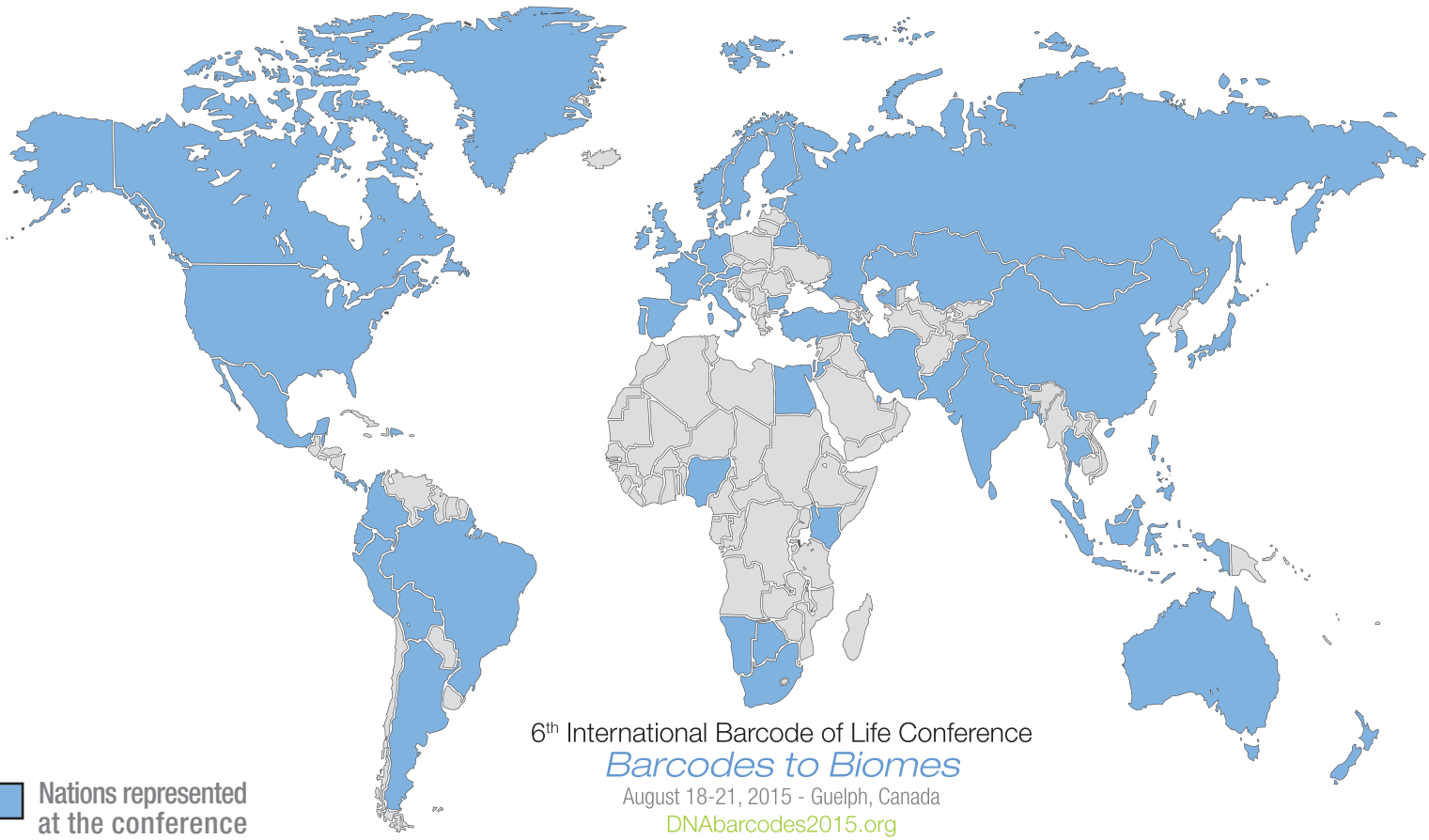
In this study, we established a barcode database for larvae of an eastern Sumatran peat swamp and we verified the validity of the barcoding approach for peat swamp larval identification. Through the DNA barcoding technique, 66% of larvae were identified to species level. The lack of success for the remaining larval specimens could be due to either an incomplete reference library or a high level of diversity, newly revealed by means of DNA barcoding.

The question concerning the possible existence of species captured as larvae but unknown for adult peat swamp communities remains unanswered as no additional species collected from the adult communities were found as larvae in that area. Hence, this investigation suggests that either the biodiversity in this particular region is much higher or the biodiversity of peat swamps has yet to be fully unraveled and portrayed.

Another interesting finding is the confirmation of a minimum of eleven species that spend their entire life in this ecosystem: *Anabas testudinae*, *Helostoma temminkii*, *Hemibagrus nemurus*, *Parosphromenus deissneri*, *Pectenocypris korthusae*, *Rasbora cephalotaenia*, *Rasbora dorsiocellata*, *Rasbora pauciperforata*, *Trichogaster pectoralis*, *Trichogaster trichopterus* and *Trichopsis vittata*. This finding explains the importance of peat swamp ecosystems for biodiversity and their particular role in maintaining the existence of at least those eleven species. In addition, increasing taxonomic resolution for larval identification will contribute to our knowledge of larval strategies, timing and dispersal, which are indisputably fundamental factors in fisheries management.

For more information on the results discussed in this article, see DOI: 10.1016/j.proche.2015.03.012

Larvae of *Trichogaster pectoralis* (above) and *Trichopsis vittata* (below) found in a tropical peat swamp.



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