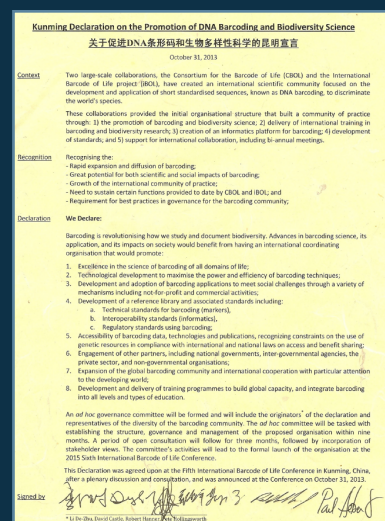




Kunming Declaration

Following discussions at the Fifth International Barcode of Life Conference in Kunming in October, 2013, the Kunming Declaration was officially signed during the conference gala. This document represents the starting point for a professional society for DNA barcoding.



Ten Years of DNA Barcoding

Transforming the way we look at biodiversity



Index:

Ten Years of DNA Barcoding (<i>Editorial</i>)	2
NorBOL Receives Infrastructure Grant	3
DNA Barcoding Contributions to IPBES	4
iBOL Colombia Advances	6
Honduran Malaise Trap Program	7
Barcoding Butterflies of Tropical Southeast Asia	9
Barcoding Bees from the South West Pacific	10
Barcoding Protists	11
BIObus 2013 Expedition	12
Flexible Ecology	13
Metabarcoding of Plantation Forests	14
DNA Barcoding Flies of Forensic Interest	15
DNA Barcoding in Hyper-diverse Malaysia	16
Barcoding Old Type Specimens	17
eThekwini Urban Barcoding Project	18
What is in the Air?	19
Top 10 Barcoding Publications (2013)	20

Ten Years of DNA Barcoding

Editorial feature highlighting key milestones in the development of DNA barcoding

Although much biological research depends upon species diagnoses, taxonomic expertise is collapsing. We are convinced that the sole prospect for a sustainable identification capability lies in the construction of systems that employ DNA sequences as taxon 'barcodes'. We establish that the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core of a global bioidentification system for animals.

This at the time rather bold statement starts off a publication which 10 years later is widely recognized as the introduction to a remarkable scientific success story. But let's begin with the prologue. Our story starts with two workshops at Cold Spring Harbor Laboratory held in 2003 to discuss the value of DNA barcoding and the feasibility of an organized effort. By many accounts the first of those workshops was not a pleasant meeting. Some taxonomists thought that Paul Hebert's idea of DNA barcoding was too simplistic. They were - to say the least - skeptical that a part of the genome could reliably serve as ID tool. This initial resistance did not vanish into thin air. "Biological identifications through DNA barcodes", currently considered a keystone publication and cited more than 2500 times over the last ten years, was initially rejected. Reviews were harsh, sometimes on the brink of being offensive, but they were just the overture to a series of anti DNA barcoding publications indicative of the strong antagonism the idea provoked.

"Intellectually bankrupt", "destructive", "a highly deficient product" that "takes away resources from other more complete research programs", "not science", the list goes on. However, where many saw limitations, others saw transformations. The idea of using a very short gene sequence to distinguish species and identify specimens immediately captured the imagination of

many scientists. Instead of being nipped in the bud, DNA barcoding was shaped through the scientific dispute and grew into a globally accepted technology with enormous potential. Only one year after the Banbury meetings, the Consortium for the Barcode of Life (CBOL) was established at the Smithsonian Institution. CBOL's main goal was to develop DNA barcoding as a global standard for species identification. Consequently, CBOL helped to organize the First International Barcode of Life Conference at the Natural History Museum in London in February 2005 and facilitated the development of international conventions such as the standard loci for major taxonomic groups.

2004 was also the year that saw the birth of an unrivaled online resource, both database and workbench at the same time. Today the Barcode of Life Datasystems (BOLD) is the leading support system for the generation and application of DNA barcode data. It is home to more than 2.7 million barcode sequences representing about 370,000 species. This is largely the result of an organic growth of national and regional networks as well as taxon and ecosystem campaigns which emerged over the years. Furthermore, the launch of the International Barcode of Life (iBOL) project in 2010 greatly increased the speed of barcode data generation. Everything is moving ahead despite those early prophecies of doom.

In October 2013, 8 years after the first conference in London, the community met again for the Fifth International Conference in Kunming, China. DNA barcoding has come a long way and what started as the vision of one man in a laboratory in Canada has turned into a poster child for funding organizations that for once turned their head away from the big 'omics' projects recognizing an idea that will transform the way we look at biodiversity.

- Continued on page 3

Ten Years of DNA Barcoding -

Continued from page 2

The story ain't over – again it was Paul Hebert who raised the bar once more. At the conference in Kunming he shared his vision of a BIN-based registry as an amendment to the current binominal naming system. Such a structure would make it possible to fully utilize the growing number of species that have been discovered with the help of DNA barcoding but are waiting for a formal description; Rod Page has called them dark taxa. Hebert compared his proposal to other systems such as the CAS registry for chemical compounds and the GSC/NGC catalog of stars and galaxies. In both instances, scientific communities used conventional naming systems for a long time before they realised that the number of potential records exceeded their capability to name them all in a reasonable timespan.

Remarkably, this time there was no strong reaction to such a provocative idea which is maybe another indication of how much the discipline has matured.

Another proof is the community's proposition to establish a formal society as expressed in the Kunming Declaration which was signed at the last conference. With CBOL redefining its mission and moving onto a more applied approach with their Barcode of Wildlife Project, there is a void to fill and the society seems a natural fit. It will also equip us with the political power necessary to finish the job we've started – building a library of the planet's biodiversity because *its assembly promises both a revolution in access to basic biological information and a newly detailed view of the origins of biological diversity*.

We have accomplished a lot in the last ten years, but there is a lot left to do.

Written by: Dirk Steinke (Editor)

NorBOL Receives Infrastructure Grant:

Project will focus on both knowledge transfer and building the barcode library

The 5th International Barcode of Life conference in Kunming was a memorable event in more than one way for the Norwegian participants. On the third day of the meeting, the Research Council of Norway announced that The Norwegian Barcode of Life Network (www.norbol.org) has been granted 25.6 M NOK (about \$4.3 M CAD) to develop the network into a national research infrastructure on DNA barcoding.



The project starts in January 2014 and will focus on knowledge transfer and capacity building in all NorBOL member institutions in addition to building a barcode library for the Norwegian fauna and flora (including fungi). NorBOL is coordinated by the NTNU University Museum in Trondheim and the network consists of 16 partner institutions throughout the nation.

Written by: Torbjørn Ekrem

DNA Barcoding Contributions to IPBES:

A tool for capacity building and knowledge transfer

Basic taxonomic knowledge and the ability to perform rapid and accurate species identification are important for sustainable development of natural resources and ecosystem services. Thus, advances in these fields should be instrumental in progressing the United Nation's Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES). Although not communicated broadly so far, DNA barcoding is a highly relevant method in studies contributing to the biodiversity assessments in IPBES.

Norway aspires to host the IPBES Secretariat on capacity building and knowledge transfer, and the Ministry of Foreign Affairs has co-funded several relevant projects in this field over the last couple of years, particularly in developing countries. One of these projects was granted to the NTNU University Museum in Trondheim late last year.

In collaboration with the La Selva Biological Station in Costa Rica, the South African National Biodiversity Institute (SANBI), the South African Institute for Aquatic Biodiversity (SAIAB) and the University Museum in Bergen (Norway), we focused on capacity building and knowledge transfer activities associated with modern tools in taxonomy. Specifically, we wanted to establish, facilitate and further develop existing north-south and south-south connections and strengthen the network between organisations and institutions that focus on taxonomy and inventory of biodiversity.

We expected that knowledge transfer would flow in all directions within the network and that the project would enhance local recruitment to the field of taxonomy for all participating countries. As such, we anticipated the project would facilitate a rapid initiation of the IPBES focus on capacity building for biodiversity studies.



Lab work at La Selva Biological Station, Costa Rica.
Image credit: Torbjørn Ekrem.

Three workshops with more than 60 participants from 16 countries were held in the period April-September 2013: one on aquatic biodiversity in South Africa, one on Central American freshwater invertebrates in Costa Rica and one on West African marine invertebrates in Bergen. The Bergen workshop was also (mainly) funded by a grant to the University Museum of Bergen from the JRS Biodiversity Foundation.

A key element in all workshops was the use of molecular tools for species identification, discovery and delineation (i.e. DNA barcoding), and the participants became familiar with the methodology, its advantages and limitations. All three workshops had as an additional goal to initiate specific DNA barcoding projects in Barcode of Life Data Systems (BOLD) and produce datasets that could be further developed and expanded after the meetings. This approach proved successful and the participants are now able to follow the progress of their samples online and continue to practice and apply what they learned at the workshop.

- Continued on page 5

DNA Barcoding Contributions to IPBES -

Continued from page 4



Distribution of more than 1000 samples prepared for DNA barcoding at the Bergen workshop. Image credit: Endre Willassen.

It was a goal for the project to improve the dialogue between the producers and users of biodiversity information in the respective regions. The project partners were key institutions and organisations within their regions and there were also participants from relevant nature management institutions attending the meetings. The workshops contributed to increased activity, regional collaboration and increased knowledge on the applied use of DNA barcoding in nature management.

Organizing international meetings like these can be challenging and the partners in South Africa, Costa Rica and Bergen did an impressive job in getting it all together and making the workshops successful events. Two issues that both relate to border control were especially challenging. Firstly, it proved difficult to arrange permits for the transport of specimens between countries in Central America. Thus, participants



Participants in the Costa Rican workshop. Image credit: Torbjørn Ekrem.

from outside Costa Rica were unable to work with specimens from their home nation at the workshop. Luckily, collecting and export permits were arranged for Costa Rica so we had enough specimens to work with at La Selva. Secondly, obtaining a visa to visit Norway was a complicated and time-consuming process for some participants from Africa. They typically had to travel long distances to the nearest embassy and spend several days away from home to obtain the documents.

Despite these obstacles, we regard the meetings as very rewarding and fruitful events. Sincere thanks in particular to Tuuli Mäkinen (SAIAB), Carlos de la Rosa (La Selva), Endre Willassen (University Museum, Bergen) and Elisabeth Stur (NTNU University Museum) for their efforts!

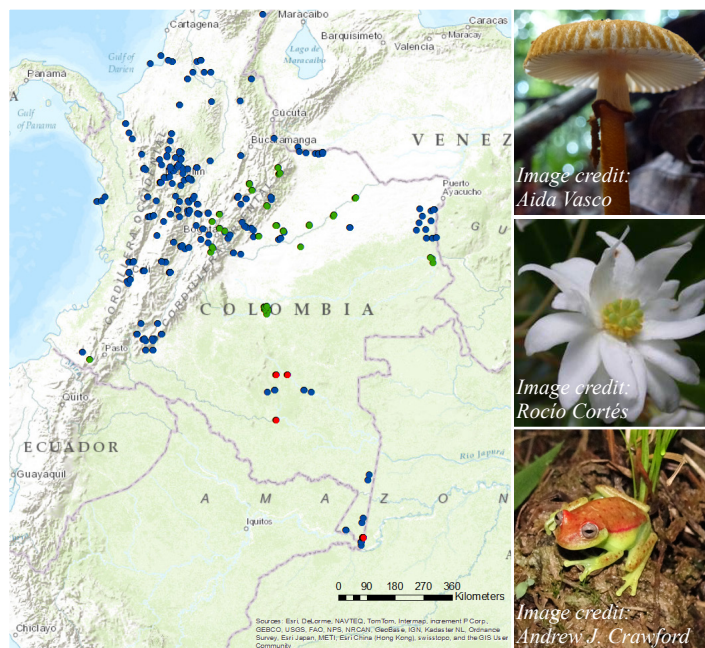
Written by: Torbjørn Ekrem

iBOL Colombia Advances:

Barcoding life in a megadiverse country

Colombia hosts an outstanding array of biodiversity, harboring the greatest diversity of birds, amphibians, freshwater fishes, butterflies and orchids of any country on Earth. Still, huge regions of the country are poorly known, and many species remain to be discovered and described. In this context, a DNA barcoding initiative represents an excellent opportunity to catalogue such a life treasure. The iBOL Colombia network (iBOLColombia.org) was officially created in 2010 and thus far comprises research projects from eight universities, three institutions of the national environmental system (SINA) and one private research center. Our aim is to promote the generation of DNA barcodes from Colombia's biodiversity as a way to support knowledge sharing and to strengthen national and international collaborations while generating a tool for environmental management.

In 2012, an IDRC small grant, managed by the Biodiversity Institute of Ontario, supported the first team work of the iBOL Colombia network with the project: "DNA barcode library of Colombian endangered flora & fauna and species of socio-economic importance". The goal was to generate 1000 DNA barcodes of plants, birds, amphibians, reptiles, butterflies and fungi as representatives of emblematic and threatened ecosystems. In addition, we selected species involved in food security or illegal trafficking such as birds listed in the CITES appendices, and several coca species and/or varieties. We used specimens from national biological collections and the laboratory processing was carried out in Colombia in order to strengthen our national technical capacity. The geographical coverage of the project within Colombia was very wide. The project involved four universities, Universidad de los Andes, Universidad de Antioquia, Universidad Distrital and Universidad Nacional sede Medellín, and two SINA institutes, the Instituto amazónico de investigaciones científicas SINCHI and the Instituto de investigación de recursos biológicos Alexander von Humboldt.



Examples of some barcoded species and geographic coverage of all specimens barcoded so far through iBOL Colombia with plants in green, fungi in red, and animals in blue.

For a country with an estimated number of species at 55000, and more than 2000 endemic species, further work is needed to generate a comprehensive DNA barcode database. We rely on the valuable contribution of our biological collections such as the tissue biorepository of the Instituto de investigación de recursos biológicos Alexander von Humboldt that hosts over 15000 samples of Colombian species. In July 2013, new decrees recognized that DNA barcoding, among other molecular techniques, are critical steps for curating biological collections, and thus do not require a special permit to be generated. This new procedure will certainly accelerate the generation of molecular information about Colombia's biodiversity. The involvement in iBOL Colombia of institutions belonging to the National Environmental Systems and of members of the most prestigious universities will ensure the reliability of DNA barcodes generated and its influence in the management of Colombia's biodiversity.

Written by: Maily Gonzalez and Andrea Paz

Honduran Malaise Trap Program:

DNA barcoding reveals extent of arthropod biodiversity

There is no simpler way to collect a large number of arthropod specimens than through the deployment of a Malaise trap. However, past use of this trap has confronted a problem – how to identify the specimens involved? The once impossible task of examining all specimens has now gained a solution - DNA barcoding. For areas with incredibly high biodiversity, such as Parque Nacional Cusuco in Honduras, this approach enables a true understanding and characterization of diversity with incredible accuracy.

Empowered by DNA barcoding, an effort to gain a detailed understanding of arthropod diversity patterns was initiated by the Global Malaise Trap Program in 2012. This program currently involves carefully directed and standardized sampling of arthropod diversity at 30 sites around the world including Honduras. A Malaise trap is a tent-like apparatus which traps insects as they fly into or crawl up the tent wall before being funnelled into a collection bottle attached to its highest point. Malaise traps were adopted by this program because they are highly effective sampling devices for numerous insect groups. As with all collection methods, only a portion of total arthropod diversity is sampled, however it represents a significant fraction of overall biodiversity.

Within Honduras, this program was supported by Operation Wallacea, an organization that designs and implements biodiversity and conservation management research programmes in various countries around the globe. During the summer of 2012, Malaise traps were deployed in Parque Nacional Cusuco, Honduras, in two different areas of broad leaf forest within the research sites Base Camp and Guanales. These sites were roughly 2 km apart with a 300 m elevation difference. Samples were collected for 8 weeks from mid-June to mid-August and subsequently analyzed at the University of Guelph.

In total, 5355 Honduran specimens were barcoded within a two month period at a cost of roughly \$2 each. Samples from both sites in the Parque Nacional Cusuco displayed a similar composition of the three major arthropod classes: Insecta (93%), Arachnida (3%) and Collembola (4%). As well, they showed congruence in their catch of insect orders with five groups dominating: Diptera (56%), Hymenoptera (20%), Coleoptera (5%), Lepidoptera (5%) and Hemiptera (4%). The barcode results revealed startling diversity – the 5355 specimens from the park included 1733 unique species. The collections from Base Camp and Guanales included 564 and 1277 species respectively. Most interesting, despite their small separation in distance and elevation, there was just 6% species overlap between the two sites! The majority of overlapping species were insects (90%) of which Diptera (61%) dominated. Coleoptera (15%), Hymenoptera (13%) and Lepidoptera (6%) showed significant overlap whereas no overlap was observed within Hemiptera (Figure 1).

- Continued on page 8



Honduran Malaise Trap Program -

Continued from page 7



Parque Nacional Cusuco is a 23,400 ha protected area in the Merendon Mountains of northwest Honduras, part of the Meso-American biodiversity hotspot, and recently identified as containing exceptionally irreplaceable biodiversity. It consists of a complex landscape with elevation ranging from 60 m to 2242 m. If sites just two kilometers apart show such great divergence in their species composition, one can only imagine the full richness of the park given the heterogeneity of the landscape as a whole.

The DNA barcodes generated by the 2012 Malaise Program within Honduras represent some of the first records of arthropods for the country. As a consequence, they will play a crucial role in the creation of an online reference library. Furthermore, this preliminary work will establish the foundation for a new method of ecosystem management within the country. As technology advances, wide-scale screening of environmental samples will be possible at low cost, allowing direct measurements of biodiversity. Such samples will be queried with the reference library in order to build a reasonably comprehensive picture of the regional dynamics of biodiversity.

Honduras faces devastating rates of loss to irreplaceable forests that are home to remarkable diversity. By harnessing the power of DNA barcoding, the rate of

biodiversity discovery, description and management can be hugely accelerated for the benefit of Honduran citizens and aiding preservation of the diversity of life on our planet.

Written by and images by: Michelle D'Souza

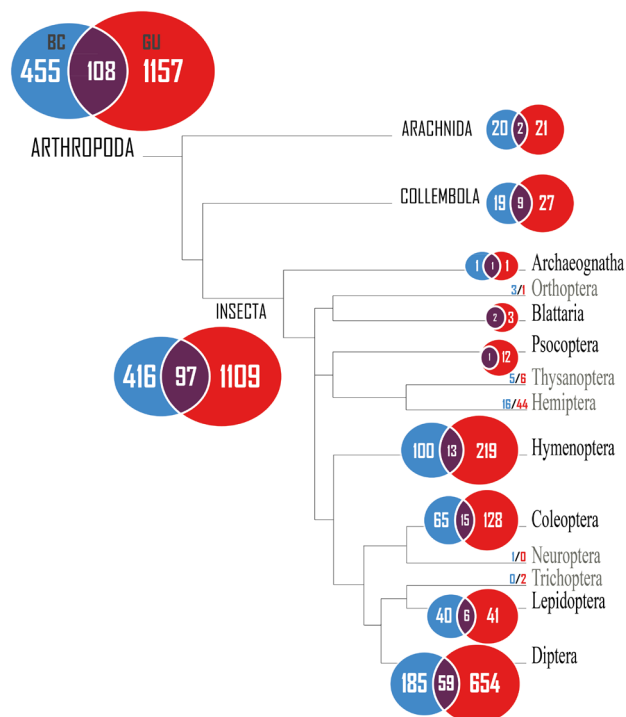


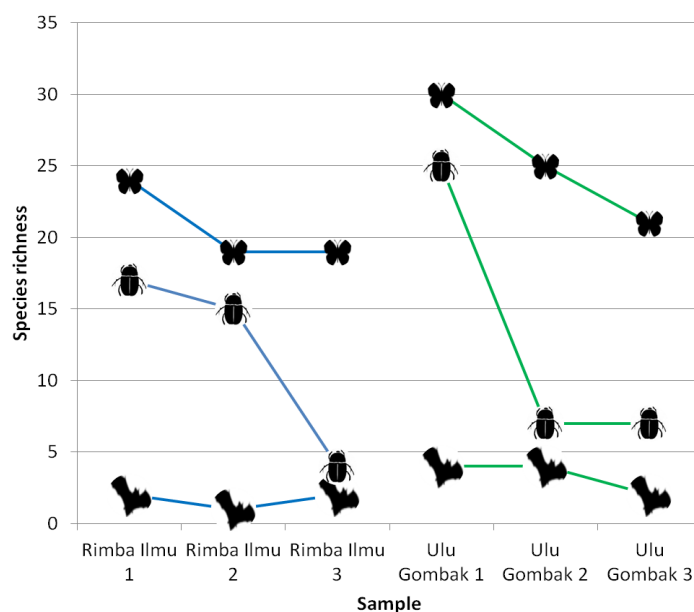
Figure 1: Frequency of species overlap (purple) between Base Camp (BC:blue) and Guanales (GU:red). Species numbers indicated on tree branch (BC/GU) for taxa displaying no overlap. Tree representing the best current estimate of arthropod relationships.

Barcoding Butterflies of Tropical Southeast Asia:

Potential as biodiversity indicators in a hyper-diverse region

Over the past 50 years, Southeast Asia has suffered the greatest losses of biodiversity of any tropical region in the world. Located at the region's centre, Malaysia is a hyper-diverse country with a particularly acute taxonomic impediment. DNA barcoding offers great promise as a solution to overcome the challenges of biodiversity monitoring and documenting the incredible diversity of the region before it is potentially lost forever. Peninsular Malaysia, in particular, has seen rapid development and changes in land use, and there is urgent need for biodiversity monitoring. For monitoring, a small group of species is frequently used as a proxy for "total" biodiversity as performing an inventory of all the species present at a site is impossible. Various attributes required by biodiversity indicator groups have been suggested but invariably include four key criteria: 1) easily surveyed, 2) tractable taxonomy, 3) broadly distributed higher taxa but specialized species, and 4) diversity patterns reflected in other groups.

Standardized butterfly surveys are time and cost-effective using sweep nets along Pollard walks or with simple fruit-baited traps. Taxonomic study of the butterflies of the region dates back to 1882 and a DNA barcode library for the 'true' butterflies of peninsular Malaysia has recently been developed, enabling rapid and accurate species identification using legs plucked from subsequently released individuals, without affecting their survival or mating success.



Butterflies can be found all over the peninsula, but show different species composition depending on ecological factors such as elevation and season. Butterfly species richness was correlated with dung beetle species richness and bat species richness at various field sites (see graph above), supporting previous studies showing a correlation between the species richness of butterflies and birds.

When evaluated against the four key criteria, butterflies show high potential as a biodiversity indicator group. We propose the initiation of a long-term butterfly monitoring scheme incorporating transects across Peninsula Malaysia and suggest butterfly surveys be given more prominence during biodiversity evaluation at sites throughout Southeast Asia.

Written by and images by: John-James Wilson and Kong-Wah Sing



Barcoding Bees from the South West Pacific:

Insights into complex population demographic histories

The South West Pacific (SWP) islands have a depauperate bee fauna, largely comprised of only three families, Halictidae, Megachilidae and Apidae. However, the taxonomy of this fauna has not been well understood because most of the species descriptions are old and spread over diverse and often obscure journals. The only archipelago for which there is a comprehensive taxonomic treatment is New Caledonia. Furthermore, taxonomic revisions for most of the bee faunas from South East Asian and the Indo-Malayan regions are also lacking. DNA barcoding therefore represents an effective strategy for documenting bees in the SWP and examining their relationship to faunas in adjacent regions.

Scott Groom, Mark Stevens and Mike Schwarz from Flinders University and the South Australian Museum have been conducting widespread DNA barcoding of bees from Fiji, Samoa and Vanuatu over the last three years. Funding for the project came through an Australia Pacific Science Foundation grant and an Endeavour Fellowship award to Scott Groom.

The results indicate that most of the apid and megachilid bees in these three archipelagos represent human-aided dispersals, predominantly from the Asian region, but also from Australia and the New World. These multiple recent introductions have the potential to change pollination dynamics in the SWP and this is an issue that will require future attention.

By DNA barcoding very large numbers of endemic halictine bees, they were also able to use mitochondrial haplotype diversities to examine historical population demography of this key regional element. They found that for all three archipelagos, halictine bee clades are relatively young, less than half a million years at

most, and that all three faunas underwent strong population declines during the last glacial maximum, but population sizes then increased dramatically with the subsequent re-warming of the current inter-glacial. However, they also found that the most basal clades were largely restricted to very high elevations, and these species may be vulnerable to future global warming. These insights would not have been possible using morphology-based systematics. The results also point to the utility of DNA barcodes for inferring complex population demographic histories, provided that sample sizes are large enough to encompass sufficient intra-specific haplotype diversity.

We can now efficiently use the biodiversity inventories of BOLD and DNA barcoding to reveal the intricate structure

of ecological communities. The next step will be to use these structures to understand the functional mechanisms both at the level of ecological rules and animal behavior and at the level of evolutionary relationships and genomic selection.

Written by: Mike Schwarz, Scott Groom, and Mark Stevens
Images by: Scott Groom



Homalictus fijiensis, an endemic short-tongued bee from Fiji.



Amegilla sp., barcodes indicate this is a recent introduction from Australia.

Barcoding Protists:

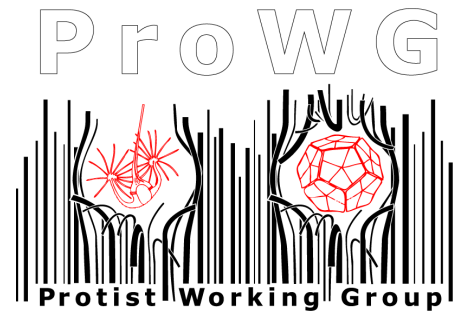
The unseen eukaryotic majority

The mainstream of barcoding activities focuses on molecular identification of animals, plants and fungi. The remaining eukaryotes, representing a large variety of protistan lineages, have received rather limited attention. Because of their commonly small size and relative paucity of distinctive morphological features, protists are usually overlooked in biodiversity surveys. Compared to the hundred thousands of animal and plant species, the $\pm 75,000$ formally described species of protists seem marginal. Yet, as pointed out by recent high-throughput environmental DNA surveys, this number could be considerably higher.

Over the last few years, a massive flow of environmental 18S rDNA data has revealed the extraordinary richness of microbial eukaryotes. In poorly explored ecosystems, up to 80% of rDNA sequences cannot be confidently assigned to any taxon available in reference databases, with whole groups of sequences forming novel lineages of morphologically uncharacterized eukaryotes.

Preliminary results from the Tara-Oceans expedition (2009-2012), aiming at assessing the biodiversity of the world marine plankton, unveiled ± 1.5 million protistan rDNA barcodes in the sunlit layer of the global ocean, a lower limit of actual species diversity for this relatively poor ecosystem in terms of biodiversity. Significantly higher richness of protistan taxa is expected in oceanic depths, either floating in the water column or crawling on the deep-sea floor. If we add to this number the free-living protists inhabiting coastal marine systems (mangroves, reefs, etc.), freshwaters and soils, as well as the protistan parasites and symbionts of animals and plants, the diversity of protists should greatly exceed that of their larger eukaryotic cousins.

Describing such a huge and poorly visible diversity is indeed a challenging task. As shown by the example of the new phylum Picozoa (former Picobiliphyta), known for a long time only as a large cluster of eDNA



sequences, it took more than six years to successfully cultivate and formally describe the morphology and ultrastructure of one species within this picoeukaryotic lineage. To do the same for all new eDNA lineages would take centuries, especially because the large majority of protists is still not amenable to culture conditions. DNA barcoding may well be the only efficient way to assess the immense diversity of protistan taxa.

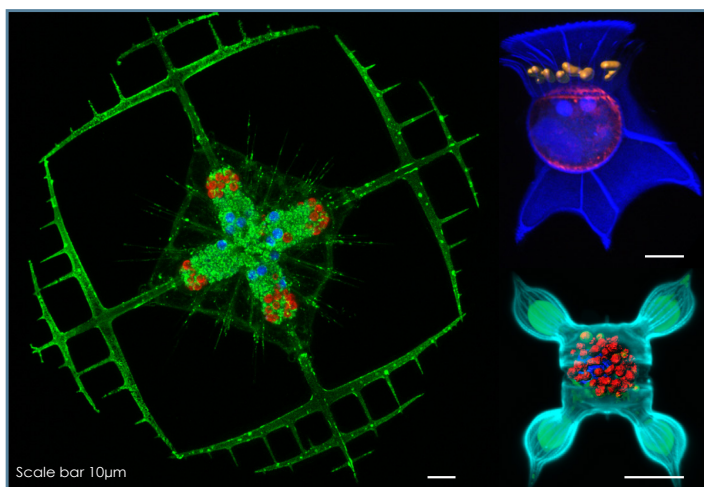
In order to facilitate this process, the CBOL ProWG (Protist Working Group) was established in 2011, with the main goal to define the most accurate DNA barcodes for all protistan lineages. Thirty experts in protist taxonomy reported on the state of the art in protist barcoding and proposed recommendations for its future development. It was agreed that the use of a single DNA barcode for all protists is not possible because of the wide genetic diversity characterizing the many protistan phyla that emerged before the origins of animals and plants.

In order to both find a taxonomic path across the immense eukaryotic domain and reach the appropriate resolution to define taxa at species level, the ProWG proposed a two-step barcoding approach. Thus, the future protistan DNA barcode reference database should contain two interconnected layers, a first holistic one including sequences of a universal eukaryotic pre-barcode, and a second group-specific one associating all pre-barcodes to a series of taxon-specific barcode designed by the specialists of a given group. The hypervariable region V4 of the SSU rDNA was chosen as the universal eukaryotic pre-barcode, while group-specific barcodes included the COI, the D1-D2 region of LSU rDNA, the ITS rDNA, the *rbcL*, the *SL* RNA, and selected regions of SSU rDNA.

- Continued on page 12

Barcoding Protists -

Continued from page 11

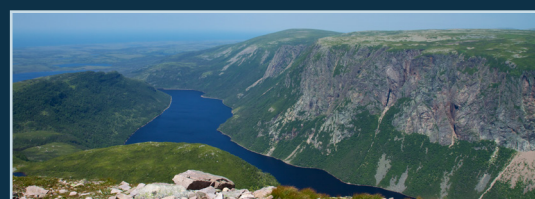


Marine planktonic protists imaged by fluorescence confocal microscopy: an acanthorean (left; *Lithoptera*), a dinoflagellate (top right; *Ornithocercus*), and a diatom (bottom right; *Chaetoceros*).
© 2013 S. Colin, EPEP/SBRoscoff.

Beyond standardization of molecular barcodes, the ProWG promotes the use of combined single-cell imaging and sequencing technologies to automatically DNA barcode and provide critical morphological information from single protists collected in the field. Inspired by the turbo-taxonomy approach, a semi-automated confocal microscopy system for high-throughput morpho-genetic characterization of single protistan cells has been proposed.

Written by: Jan Pawlowski and Colomaban de Vargas, co-chairs of CBOL Protist Working Group

BIObus 2013 Expedition: Exploring the biodiversity of eastern Canada



Sampling focused in 14 National Parks

- | | |
|---------------------------------------|---|
| 1. Pukaskwa National Park | 8. Terra Nova National Park |
| 2. Georgian Bay Islands National Park | 9. Torngat Mountains National Park |
| 3. Rouge Park/Toronto Zoo | 10. Fundy National Park |
| 4. La Mauricie National Park | 11. Kejimikujik National Park |
| 5. Mingan Archipelago National Park | 12. Cape Breton Highlands National Park |
| 6. Forillon National Park | 13. Prince Edward Island National Park |
| 7. Gros Morne National Park | 14. Kouchibouguac National Park |

BIObus Statistics 2013:

- # of kms driven: **16766**
- # of tire blowouts: **0**
- # of BIObus blog entries: **44**
- # of public engagement events: **11**
- # of Malaise traps deployed: **82**
- # of lots collected: **1538**

Flexible Ecology:

DNA barcoding and molecular food webs

Steady progress in the expansion of the Barcode of Life Datasystems (BOLD) and DNA barcoding as a method over the last decade means the science and the resources have reached a level of maturity that allows for exciting new ecological applications. One of these is the rapid growth of dietary studies and food web analyses that rely on both DNA barcoding and BOLD. This is particularly true now that “next generation sequencing” has become a simple and quick way to plough through gut contents and faeces recovering partial DNA barcodes from consumed and digested prey.

Several research groups have been combining efforts in the UK to look at food webs in a variety of locations around the world. For the most part we have been working with insectivores but we are rapidly branching into investigations of seed dispersal, pollination and herbivores. This has included projects related to the coexistence of ensembles of insectivorous bats like those living in caves in Jamaica, the ability of some species to switch between trophic levels like the Pallas’s long tongued nectar bat which turns out to be a voracious insect hunter and the coexistence of endangered and invasive species competing for resources such as the endemic skinks and invasive shrews of Round Island off the coast of Mauritius.

In some areas, like Ontario, Canada, the robustness of the database allows us to identify fragments of

DNA barcodes recovered from faeces under roosts to reconstruct the movement of bats between habitats and to evaluate the quality of those habitats based on prey which act as environmental indicators. We do not even have to see the bat, the prey or the act of foraging. It’s like looking through your neighbour’s garbage to see where they shop!

In other areas, like the Philippine islands, we rely on classifying barcodes as Species 1, Species 2, Species 3, etc. but we can still make specific observations about the level of overlap between the diet of competing species.

We have learned many important lessons about how to collect the data and how to interpret food webs with this new species-level of resolution. However, the most amazing thing we have found across dozens of studies is how incredibly flexible each system and each predator appears to be.

We can now efficiently use the biodiversity inventories of BOLD and DNA barcoding to reveal the intricate structure of ecological communities. The next step will be to use these structures to understand the functional mechanisms both at the level of ecological rules and animal behavior and at the level of evolutionary relationships and genomic selection.

Written by and images by: Elizabeth Clare



Reconstruction of food webs through the DNA barcoding of faeces has shown that an ensemble of bats in Jamaica divides up resources according to partitioning by morphology, echolocation and hunting time.

Metabarcoding of Plantation Forests:

Informing management decisions for sustainable ecosystems

How can ecosystems be best managed for biodiversity? Without access to detailed and comprehensive biodiversity data to tell us how communities respond to management actions, policy makers have been forced to rely on little more than intuition for guidance. However, funding for conservation is limited and our efforts to halt the biodiversity declines have been largely unsuccessful to date, so there is an urgent need for more evidence-based decision making. The development of metabarcoding has finally provided the opportunity to gather large scale detailed biodiversity data across a wide range of taxonomic groups that can provide the required evidence base to inform the sustainable management of ecosystems.

As part of my PhD with Doug Yu (University of East Anglia, UK), I conducted a pilot study in collaboration with the UK Forestry Commission to demonstrate how metabarcoding can be used to inform the management of plantation forests for biodiversity. We sampled arthropods from fifteen plantation stands in Thetford forest, UK, each of which was characterised by either a single-species crop of oak (5 sites) or Scots pine (4 sites), or a mixed crop of oak and Scots pine (6 sites). Trapping was conducted for eight consecutive weeks across all forest stands, giving a total of 120 (8 x 15) Malaise trap samples. We used '454' sequencing of the full COI barcode region, treating each trap sample as a separate MID, and followed the 'biodiversity soup' bioinformatics protocol to denoise the sequences, cluster them into OTUs, and assign taxonomy.

Our key questions were: 1) Is a mixed crop better for biodiversity than a single crop? 2) Do any commonly-used structural indicators correlate with species richness or explain species composition of flying arthropods? 3) In general, what is the best strategy for maximising biodiversity at the landscape scale?

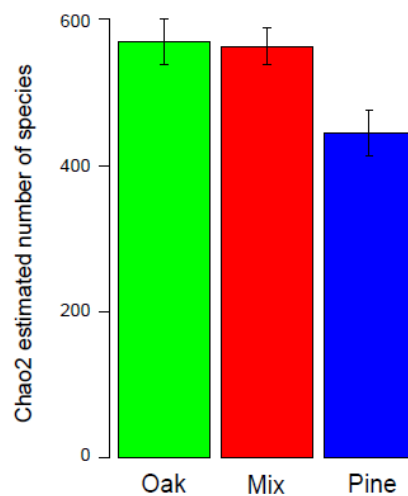


Figure 1: Estimated total species richness for each crop type (Chao2 incidence coverage method).

Contrary to expectations, we found no evidence that a biodiversity benefit is derived from planting a mixed species crop compared with a single species crop; mixed crop communities were compositionally intermediate to oak and pine but were no more species rich than those in pure oak crops (Figure 1). Pure pine stands had the lowest species richness, but they contained many specialists, which suggests that eliminating all pine monoculture stands from the landscape would result in an overall loss of biodiversity. At the scale of this study, no structural indicator correlated significantly with species richness, but a combination of the conifer/broadleaf ratio, tree species diversity and plantation crop density explained 60% of the variation among sites in community composition. In general, these results support management to provide a diversity of habitats, rather than management to provide a set of characteristics that are correlated with slightly higher species richness ("management for

diversity requires diversity of management"). We also uncovered a surprisingly rapid rate of species turnover over the eight trapping weeks, which emphasises the importance of controlling for sampling date when designing experiments of this type.

Written by and images by: Catharine Bruce

DNA Barcoding Flies of Forensic Interest:

Validation test using local entomofauna

Fly larvae and pupae collected on corpses may be used to draw conclusions when investigating legal cases. For example, the minimal time since death (also called post-mortem interval or PMI) can be estimated using species whose developmental times are known for different temperature conditions. The correct identification of forensically important flies is essential in forensics because biological traits (such as ecology or developmental times) can vary among species, even when these are closely-related. In this context, DNA barcoding can be very useful because it can provide species level identifications with an excellent resolution and it is applicable to larvae and pupae.

Although DNA barcoding has been validated for forensic use, the reliability of the reference library of DNA barcodes for identifying insects collected locally has to be verified. This has significant legal importance because local species (or diverging populations) that are not represented in the reference library may be misidentified by DNA barcoding. Therefore, DNA barcoding of local flies of forensic interest has a triple interest: 1) It can be used to validate reference libraries of DNA barcodes for specific geographic areas. 2) The reference library can be extended with additional reference barcodes that were not yet represented in the reference library. 3) Misidentified reference sequences might be detected and commented in cases where inconsistent identifications are found and voucher specimens have been re-examined.

Flies of forensic interest collected on Belgian and French crime scenes have been DNA barcoded in a collaborative project involving the Royal Museum for Central Africa (RMCA), the Royal Belgian Institute of Natural Sciences (RBINS) and the National Institute of Criminalistics and Criminology (NICC) of Belgium. The results of this small DNA barcoding project (85 specimens, seven species of Calliphoridae, six of Muscidae and three of Fanniidae) provided 24 barcode sequences that were not yet represented in the reference

library of the Barcode of Life Data Systems (BOLD) and provided barcodes for two species of forensic interest that had not yet been barcoded.

Our validation test evaluated the reliability of the Identification System (IDS) of BOLD to correctly identify the specimens. Indeed, BOLD yielded correct identifications for all queries belonging to species that were represented in the reference library. Using the dataset of BOLD including public records and early-released sequences (Species Level Barcode Records) increased the number of correct identifications. However, a large proportion of these identifications (50% of the queries) were ambiguous (correct identifications together with incorrect ones). Each of these ambiguous identifications could be resolved 1) by adapting the search method (e.g. only considering the most similar sequences to distinguish closely-related species), 2) by correcting sequences of the reference library that are most probably mislabelled or 3) by using additional molecular markers when species share barcodes. Each of these observations can be shared with other users by tagging or commenting the appropriate records in BOLD.

- Continued on page 16



Larvae on corpse. © 2013 Luc Bourguignon, Microtraces lab, NICC.

DNA Barcoding Flies of Forensic Interest -

Continued from page 15



Fly of forensic interest, *Cynomya mortuorum*.
© 2013 Erena Dupont, Microtraces lab, NICC.

In conclusion, we found that the BOLD platform offers appropriate tools to improve the quality of the reference library of DNA barcodes for forensic use. Indeed, it allows the annotation of the records used as reference data and their continuous quality control (e.g. published or early-released records, barcode compliant or not, link with voucher specimens, “red flagged” by other users, etc.).

Written by: Gontran Sonet

DNA Barcoding in Hyper-diverse Malaysia:

Seminar and workshop lay foundation for Malaysia Barcode of Life network

In order to promote DNA barcoding in Malaysia the University of Malaya in Kuala Lumpur (UM) hosted the “father” of the movement, Paul Hebert, as a Visiting Professor in October 2013.

A public seminar by Paul on the International Barcode of Life was attended by more than 200 people representing numerous academic, government, NGO and research institutions from across the country. A “basics of DNA barcoding” laboratory workshop introduced 20 graduate students and young scientists to the simple molecular techniques required for biodiversity genomics research. A scientific meeting for professors, lecturers and government researchers was held to instigate the activation of Malaysia as an iBOL partner nation.

In addition to the public events, discussions with the university’s top administration were very fruitful. Details are being finalised for the creation of a high-throughput DNA barcoding centre and Malaysia Barcode of Life network spearheaded by UM, capable of leading the region towards heightened biodiversity consciousness.



Written by and images by: Kong-Wah Sing and John-James Wilson

Barcoding Old Type Specimens:

Unravelling taxonomic mysteries

When a new species is discovered, a single specimen is assigned as the “holotype”, which can be thought of as the official example of that species. Any other specimen thought to belong to that species must match the holotype (or “type specimen”) in terms of both morphology and genetics. Morphology can be difficult to assess, particularly in cases of cryptic species complexes where many species appear physically identical. The recovery of barcode data from type specimens is critical for the resolution of cryptic species complexes, as well to ensure the appropriate use of names for contemporary specimens. In cases of rare, extinct, or extirpated species, type specimens may be the only source of genetic material, making them invaluable for any barcode library construction.

Type specimens have been barcoded in the past, but those studies usually only included type specimens from one or two species. A large-scale type specimen barcoding project was initiated in late 2012 (with support from the Gordon and Betty Moore Foundation) with the goal of developing an inexpensive and effective means of recovering barcode data from type specimens ranging in age from 50-200 years. Initial testing is focused on about 4000 specimens, most belonging to two families of Lepidoptera - Geometridae and Xyloryctidae - but also other lepidopteran families, giant lacewings (large, elusive neuropterans) and several bat species. The refinement of protocols harnessing ancient DNA techniques combined with a medium-throughput platform has enabled the recovery of 100-200 bp sections of the COI barcode region from most individuals.



Example of a pinned type specimen.

© 2013 Marko Mutanen, University of Oulu.



Ancient DNA lab at the Biodiversity Institute of Ontario. Image credit: Kellyn Hawley.

Although the sequences are short, they are, in most cases, sufficient to enable their unambiguous connection to one representative of a particular species complex. In fact, initial results have been used to unravel several cryptic species complexes. For example, the barcode sequence recovered from the holotype of a moth species found in Papua New Guinea and Australia showed that only the Papua New Guinea specimens are

appropriately named - the Australian specimens are a different species that will need to be named. Similarly, results from bat type specimens are calling into question century old taxonomic beliefs.

In addition to the practical applications, analysis of factors affecting the recovery of type specimen barcodes was also possible due to the availability of metrics such as sample taxonomy, age, size, tissue type, and tissue treatment. Not surprisingly, taxonomy and sample size (i.e. the amount of tissue used to extract DNA) greatly affected the likelihood of recovering DNA from a type specimen. On the other hand, age, tissue type (dry legs vs. full abdomens) and tissue treatment (grinding tissue to release more DNA vs. not grinding) were not found to significantly affect success rates (despite the fact that one would expect more DNA from young samples, full abdomens, and ground tissues). Further analysis of these factors are underway, as well as experiments harnessing the power of next generation sequencing for the rapid recovery of full barcode sequences (658 bp) from type specimens.

Written by: Sean Prosser

eThekwini Urban Barcoding Project:

DNA barcoding explores invertebrate diversity in an urban setting

Rates of urbanization in Africa are the highest in the world. By 2025, more than half of the African population will be urban. Factors associated with urbanization such as habitat loss, climate change, pollution, species invasions and overexploitation are all major components of biodiversity loss. All of these factors contribute towards urban areas generally having lower biodiversity when compared to natural areas.

The eThekwini region is a South African metropolitan municipality covering a land area of 2297 km² including the coastal city of Durban (29.8833°S, 31.0500°E) and surrounding towns. Although eThekwini is located within the Maputaland-Pondoland-Albany biodiversity hotspot, the area is heavily urbanized. In addition, the Durban Harbor is the largest and busiest shipping terminal on the African Continent and is a major source of potentially invasive species entering the country. To integrate and conserve areas of high biodiversity within the city, a network of open spaces has been established, linking nature reserves and undeveloped pieces of privately and municipality-managed land. This network of natural habitats within the city acts as reservoir for local diversity. Exactly how many species of plants and animals (endemic and exotic) occur in these “green spaces” is not clear.

As a first step towards an all-taxa biodiversity inventory for eThekwini, the Urban DNA barcode project was started in 2011 as a collaboration between the University of KwaZulu-Natal and the eThekwini Environmental Planning and Climate Protection Department. Invertebrate taxa, including keystone species such as bees, flies and spiders, were collected from several open spaces within the city of Durban using a standardized protocol; to date 19 open spaces have been sampled.

Few DNA barcoding studies have focused exclusively on biodiversity within urban environments. This study uncovered unexpectedly high species richness in the

urban environments surveyed. Of the 8265 invertebrate specimens submitted for barcoding, barcode compliant sequences were recovered from 7065 specimens, the majority of which represent species new to the Barcode of Life Datasystems (BOLD). For the charismatic insect order Hemiptera (true bugs), for example, DNA barcode data from 1032 specimens representing 312 BINs were added to BOLD; 92% of these records were new to BOLD (less than 95% sequence similarity with other records). Perhaps the most surprising result is that less than 10% of the records could be assigned to introduced or invasive taxa, suggesting that open pieces of land within the city are not depauperate wastelands but provide important refugia to endemic taxa.

In the future, with the support of the eThekwini municipality, we hope to extend our sampling by encouraging citizen scientists across the municipality to sample invertebrate fauna from their local “green spaces” and send them in for barcode sequencing. These data, combined with climatic data collected by the municipality, could in the long term provide a valuable environmental monitoring tool and will be used to better understand what impact urbanization is having on indigenous biodiversity.

Funding provided by eThekwini Municipality through the KwaZulu-Natal Sandstone Sourveld Research Programme and by the International Development Research Centre, Canada.

Written by: Sandi Willows-Munro



Images credit: eThekwini municipality

What is in the Air?

Using DNA barcodes to detect indoor fungal composition

Indoor human environments contain a variety of microbes, some of which are detrimental to human health. Problems develop in damp buildings, where various materials become wet for extended periods of time. Such dampness provides the moisture that supports the growth of bacteria and fungi (i.e. mould). Mould in buildings is positively associated with several allergic and respiratory issues, and certain moulds are toxigenic, meaning that they can produce dangerous mycotoxins. The significance of this phenomenon is striking. Recent media attention has increased public awareness and concern over exposure to moulds at homes, in workplaces, schools etc.

Indoor fungi are traditionally determined by culture-dependent methods, which inevitably have a low resolution, underestimate diversity and are biased towards fungi that grow well on generic growth media and produce characteristic morphological structures allowing identification. To increase precision of indoor fungi analyses and to provide useful data for end-users, we developed a project, for which we collected fungal samples from buildings with a known or suspected mould problem (two kindergartens) and from control buildings, and conducted DNA barcoding (nuclear ITS region) using next generation sequencing methods allowing efficient metabarcoding of fungal samples. Problem buildings were examined both before and after renovation, and also seasonal variation in fungal diversity was considered.

Sampling was conducted using a collector with a disposable filter attached to the tube of a vacuum cleaner. Multiple horizontal and vertical samples were collected from each building included in the study. After vacuuming, the filter containing the dust was removed from the collector and placed in a plastic bag until processing, which included cutting the filter, rinsing it with water and emptying the content into a petri dish, where large non-biological particles were removed. Subsequently, the sample was grinded and DNA extracted, followed by DNA amplification and sequencing.

The results obtained so far suggest that DNA barcoding provides high resolution in fungal identification and allows for a more comprehensive discovery of taxonomic diversity when compared to culture-dependent methods. The number of fungal genera per sample varied from 13 to 124. Considerable variation in fungal composition occurs even within the same building, which emphasizes the importance of multiple sampling. We also found that taxonomic diversity of fungi is not a good indicator of indoor air quality - a diverse array of fungi occurs even in a normal indoor environment. However, the presence and dominance of fungal taxa known to cause allergic and respiratory effects and/or being indicators of moisture damage are better indicators of air quality. Once the on-going renovations of the problem buildings are over, it will be highly interesting to see how the fungal composition and diversity has changed and how successful the renovations have been.

The work is conducted at the University of Helsinki, Finland in collaboration with the City of Helsinki and is funded by Marjatta and Eino Kolli Foundation.

Written by and images by: Helena Korpelainen



Top 10 DNA Barcoding Publications 2013

Measured using Publish or Perish (Jan-Dec)

Metrics are largely based on Google Scholar ranking and journal access statistics.

1. Santoferrara LF, McManus GB, Alder VA (2013) Utility of genetic markers and morphology for species discrimination within the order Tintinnida (Ciliophora, Spirotrichea). *Protist* 164: 24-36.
2. Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. *PLoS ONE* 8: e66213.
3. Souffreau C, Vanormelingen P, Van de Vijver B, Isheva T, Verleyen E, Sabbe K, Vyverman W (2013) Molecular evidence for distinct antarctic lineages in the cosmopolitan terrestrial diatoms *Pinnularia borealis* and *Hantzschia amphioxys*. *Protist* 164: 101-115.
4. Zhou X, Li Y, Liu S, Yang Q, Su X, Zhou L, Tang M, Fu R, Li J, Huang Q (2013) Ultra-deep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. *GigaScience* 2: 4.
5. Walther G, Pawlowska J, Alastruey-Izquierdo A, Wrzosek M, Rodriguez-Tudela JL, Dolatabadi S, Chakrabarti A, de Hoog GS (2013) DNA barcoding in Mucorales: an inventory of biodiversity. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 30: 11-47.
6. Baselga A, Fujisawa T, Crampton-Platt A, Bergsten J, Foster PG, Monaghan MT, Vogler AP (2013) Whole-community DNA barcoding reveals a spatio-temporal continuum of biodiversity at species and genetic levels. *Nature Communications* 4:1892.
7. García-Robledo C, Erickson DL, Staines CL, Erwin TL, Kress WJ (2013) Tropical plant–herbivore networks: reconstructing species interactions using DNA barcodes. *PLoS ONE* 8: e52967.
8. Ji Y, Ashton L, Pedley SM, Edwards DP, Tang Y, Nakamura A, Kitching R, Dolman PM, Woodcock P, Edwards FA, Larsen TH, Hsu WW, Benedick S, Hamer KC, Wilcove DS, Bruce C, Wang X, Levi T, Lott M, Emerson BC, Yu DW (2013) Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters* 16: 1245-1257.
9. Decaëns T, Porco D, Rougerie R, Brown GG, James SW (2013) Potential of DNA barcoding for earthworm research in taxonomy and ecology. *Applied Soil Ecology* 65: 35-42.
10. Murphy RW, Crawford AJ, Bauer AM, Che J, Donnellan SC, Fritz U, Haddad CFB, Nagy ZT, Poyarkov NA, Vences M, Wang W-z, Zhang Y-p (2013) Cold Code: the global initiative to DNA barcode amphibians and nonavian reptiles. *Molecular Ecology Resources* 13: 161–167.