Students Explore the Impacts of Invasive Species

Research: Developing Metabarcoding Workflows
Applications: Barcoding Pollen for Forensics

Image credit: flickr.com/photos/pacificklaus
Welcome to our September 2016 issue of the Barcode Bulletin.

This issue highlights numerous contributions from last year’s conference as many of them have recently been published in special issues. They are supplemented by stories on citizen science, forensic palynology, plant-pollinator networks, and more.

Enjoy reading,

Dirk Steinke
Editor-in-chief

Arctic-alpine species are restricted to the subarctic and arctic zone of the northern hemisphere and to the Alps and other southern mountain systems, thus showing a strong allopatric distribution pattern. They are particularly challenging examples for species delimitation due to the long isolation of populations, going back at least to the last glacial period.

A new project of the Naturmuseum Südtirol, Italy (Dr. Vito Zingerle) and the Tiroler Landesmuseen, Austria (Dr. Peter Huemer), in close cooperation with the University of Oulu, Finland (Dr. Marko Mutanen) and BIO-CBG, Canada (Prof. Paul Hebert), aims to focus on barcoding of four invertebrate groups: spiders (Araneae), grasshoppers (Orthoptera), beetles (Coleoptera), and butterflies and moths (Lepidoptera), both from the Alps and Finland and wherever possible from North America. Genetic and morphological data of a minimum of 50% of the arctic-alpine species inventory, covering about 60 species, will be evaluated to test species boundaries. Preliminary data analysis indicates that hitherto overlooked cryptic diversity is much more widespread than expected.

With an already existing barcode library for many lepidopterans from the target regions, it is planned to probe deeper by using RAD sequencing, which will be done at the facilities of one of the major partners, the University of Oulu, with support from the Finnish Academy. The major aim is DNA-based delimitation of allopatric populations by implementing a general mixed Yule coalescence model (GMYC) and a Poisson tree process (PTP model).

Thanks to this innovative approach, the project has been successfully launched and will receive the necessary financial support from the Promotion of Educational Policies, University and Research Department of the Autonomous Province of Bolzano - South Tyrol.

Written by: Peter Huemer
In the thirty years since the accidental introduction of venomous lionfish to South Florida, this highly invasive species has been able to successfully colonize and dominate sites throughout the West Atlantic and Gulf of Mexico. Lionfish can now be found in natural and artificial habitats to a depth of more than 100 meters, at sites as far north as Rhode Island and as far south as Venezuela. Lionfish prey on small invertebrates and fishes and, once established, they can reduce prey species abundance by up to 90%.

To better understand the impacts of invasive lionfish on northern Gulf of Mexico marine communities, the Gulf Islands Research and Education Center (GIREC; http://uwf.edu/girec) is partnering with local middle and high school teachers and their students to use DNA barcoding to identify lionfish prey. This partnership among the University of West Florida, Gulf Islands National Seashore, and the school districts of Santa Rosa and Escambia County represents an opportunity for students to gain hands-on experience in genetics through a series of engaging lessons aligned with state and national science standards.

University of West Florida faculty assist participating teachers with lesson development, implementation, and management; access to necessary equipment and supplies; and an undergraduate student to support in-class activities. Over a period of five classes, student teams dissect donated lionfish and then isolate, copy, and visualize COI gene sequences from dissected prey items. Students then clean and proofread their DNA sequences and identify successfully amplified prey items using the BOLD Identification System.

During the project’s first year, more than 500 participating students produced 152 scorable sequences. Protocols prioritized identification of vertebrate prey, revealing ten fish species preyed upon by lionfish. A particular challenge still to be overcome is the frequency of apparent sample contamination. Nearly 30% of the scored sequences were identified as lionfish. While double peaks in some sequence trace files clearly indicated cross-contamination, additional testing is needed to determine whether high-quality sequences identified as lionfish represent additional cases of contamination, or evidence of cannibalism (adult lionfish have been observed preying on juveniles, but the frequency of such events is unknown and may vary considerably among sites).
The majority of remaining samples (87%) comprised three species, the round scad (*Decapterus punctatus*), vermillion snapper (*Rhomboplites aurorubens*), and pearly razorfish (*Xyrichtys novacula*). All species identified by students commonly occur in the shallow coastal waters of the northern Gulf of Mexico, and together highlight potentially important consequences of the lionfish invasion.

**DNA barcodes generated by students identified ten fish species preyed on by lionfish.**

Most significantly, round scad and vermillion snapper are directly targeted by commercial and sport fisheries, indicating possible economic impacts. Lionfish consumption of many juvenile species also has the potential to reduce marine biodiversity, putting additional stress on marine communities already impacted by overfishing, climate change, and pollution.

Student-led DNA barcoding of lionfish prey will continue this coming school year. The data that the students generate will be shared among classes to give students a chance to develop and test their own hypotheses, as well as with marine resource managers to promote the development of effective strategies to reduce impacts from this highly invasive species.

*(Participating schools: Booker T. Washington High School, Escambia High School, Gulf Breeze High School, Navarre High School, Pensacola High School, West Florida High School, and Woodlawn Beach Middle School.)*

*Special thanks to: Niuhi Divers for donating lionfish, Ryan Lavoie and Jullianne Rawson for their expert in-class assistance, and to all the participating teachers for their dedication to their students.)*
One year ago, 601 participants from 51 nations gathered in Guelph, Canada for the 6th International Barcoding of Life Conference. The rich array of presentations and discussions showcased the achievements and emerging research areas in our community. Promising trends included the growth of DNA barcoding globally and in the service of diverse socially and environmentally important applications.

To mark the anniversary of the conference, a multi-part special issue of full articles is being published in Genome, which also hosts the open-access special abstracts issue from the conference. The September 2016 issue comprises part 1 of “Barcodes to Biomes”, consisting of two reviews and 10 articles. Part 2 will contain an additional 12 articles, and a large virtual special issue will be collated online in early 2017, consisting of dozens of additional conference-associated articles published across multiple issues of Genome.

The articles in part 1 represent several dominant themes of the 6th Conference. Roslin and Majaneva review how the DNA barcoding of entire communities reveals food web structure in unprecedented detail. The review by Bell et al. synthesizes topics from a special session on pollen DNA barcoding, while the article in this Barcode Bulletin by Bell et al. highlights an important application of pollen barcoding: forensic palynology.

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Two original articles focus upon the process of conducting large-scale DNA barcoding campaigns (Janzen and Hallwachs; Geiger et al.), while the works by Hausmann et al. and Mark et al. highlight how new sequencing technologies can be applied towards the fundamental task of building libraries of reference DNA barcodes. Saitoh et al. present a major step forward in quantifying specimen abundance when metabarcoding soil invertebrates. Focusing upon marine reef-dwelling invertebrates, Al-Rshaidat et al. further highlight the value of standardization in field and molecular methods for the quantification of biodiversity and for comparisons across sites and research teams.
DNA barcoding activities are now sufficiently widespread as to be the subject of a formal analysis of media coverage. Geary et al. present intriguing results regarding the reporting and framing of DNA barcoding-related news stories. They additionally reveal gaps in the coverage of complex issues such as international collaboration and the movement of genetic materials across borders.

Three other original research articles demonstrate several of the diverse ways that DNA barcoding is being used for exceptional study systems and for socio-economically important applications. Sing et al. employ DNA barcoding to expedite butterfly surveys in urban parks and to investigate the kinds of parks and microhabitats that can best promote conservation.

Focusing upon an isolated and fragile environment, Beet et al. find cryptic diversity and an interesting biogeographic history of the collembolans in Antarctica. Finally, Williamson et al. turn their attention towards a globally important conservation crisis: the extinction threat being faced by the cycads, a culturally important and evolutionarily unique life form on Earth. They use DNA barcoding to identify specimen fragments being traded in traditional medicine markets, finding examples of threatened species being illegally harvested from the wild. Such knowledge is an important step in validating product supply chains and in taking conservation actions.

Together, the articles of part 1 represent a large research community that is diverse and evolving, contributing to fundamental scientific knowledge and to the protection of natural resources of intrinsic and economic value. Enjoy, and stay tuned for part 2!
** denotes graduate student or postdoctoral prize winner at the 6th International Barcode of Life Conference

Articles in the “Barcodes to Biomes” special issue in Genome (part 1)


In the first phase of GBOL, “...a national web portal for DNA barcodes and specimen data was developed...”

Since November 2011, the German Ministry of Education and Science (BMBF) has funded a consortium of 17 natural history museums and research institutions to set up the ‘German Barcode of Life’ initiative (GBOL). The main objective was to establish a network of professionals and non-professionals to begin the construction of a DNA barcode reference library for the fauna, flora, and fungi of Germany (see also Barcode Bulletin Sep. 2014). The project nicely connected to the ‘Barcoding Fauna Bavarica’ (BFB) initiative, which has been running since 2009, building a barcode library for Bavaria, the largest state in Germany. Upon completion of the first phase of GBOL (2011-2015), the majority of the project’s goals were achieved. Among them, a national web portal for DNA barcodes and specimen data was developed and is being continuously improved (www.bolgermany.de) by over 250 citizen scientists and more than 50 institution-based taxonomists. In particular, the engagement of external experts contributed significantly to the project’s success.

The coordination of such a heterogeneous group of researchers and the data that they produced was challenging, as was the development of a common web portal, which should ideally display all material from the time that it is entered into a database. While most databases have now been integrated, and exchange protocols have been developed for local FIMS/LIMS systems, we are still working on a semi-automatic interface to connect and exchange data with BOLD. Through a newly developed GBOL ‘data broker’, we are delivering data compliant with the international ABCDDNA standard, which are harvested in xml format and kindly being included in BOLD. The exchange in the opposite direction is still under development.
Of the 48,000 animal and 10,000 plant species (excluding algae and fungi) present in Germany, over 23,000 different species plus a few selected rust fungi (Pucciniales) have been processed and DNA barcodes have been generated. In total, 295,000 specimens were submitted to GBOL institutes, and, after choosing (usually) up to 10 individuals per species from throughout their distribution range in Germany, 145,000 of them delivered a DNA barcode.

For plant species, we aimed to generate data for the official DNA barcode markers \(rbcL\) and \(matK\), but also for fast-evolving plastid DNA regions (\(trnL-F\), \(rpl16\), \(trnK/matK\)) and the nuclear internal transcribed spacers (ITS). To cope with the higher complexity of DNA sequence assembly and the generation of valid contigs for several markers with access to raw data (trace files) from different institutes, a web application has been programmed to serve the needs of the botany section (www.gbol5.de). Our results from the team working on soil fauna and limnetic nematodes indicate that DNA barcoding in these organisms would likely also benefit from the use of additional, nuclear markers, as unexpected and high diversity in COI occurred in several genera.

So far, GBOL members have given 158 presentations at national and international events, and the German media have reported on the project in 72 articles, interviews, and TV specials. Of those, the discovery of new species, invasive species, or potentially harmful species were the stories with the broadest uptake. Almost 50 scientific articles discussed results from GBOL, ranging from large data releases with many thousands of species to the notion of a rediscovered species.

As it would not be fair to highlight only a few contributions here, we direct the reader to the GBOL website or the recently compiled, official report on the first project phase, available on our website. There you will also find information on the granted, second funding period (€6 million for three years), where we are focusing on establishing DNA barcoding as a tool for practical applications, and which includes several new partners and organism groups (diatoms, fungi).

For more information, see DOI: 10.1139/gen-2015-0185.
Biologists’ interest in working out the associations between species has increased massively during the last two decades. What we have come to realize is that in order to understand how nature is built, how it works, and how it changes as a consequence of our actions, it is not enough to describe what species are where: we also need to understand how these species tie into each other. As the perhaps simplest form of an interaction between species, we may start by describing who eats whom. This basic knowledge comes with profound implications: clearly, a specialist predator cannot persist without its single type of prey. For a generalist predator, a change in the availability of different prey species may affect the amounts eaten of each. At the level of the whole ecosystem, the way species are linked with each other will dictate how the system works, and how sensitive it is to perturbation. In brief, if we want to understand nature, we need to understand its food webs.

Determining the structure of a food web may sound trivial – after all, we just need to establish the nodes, i.e. the species, and their links, i.e. who eats whom (and preferentially how often, to know the strength of the link). Yet, this task is anything but easy. A large fraction of species are too similar, too small, too soft, or too secretive for their diet to be recorded by traditional means. Others dissolve their food into goo as soon as they have caught it, leaving no fragments behind for us to identify. And together, they form communities so rich in species that in order to detect their associations, we will need to inspect enormous samples.

**DNA barcodes may serve as the new glue between aquatic and terrestrial researchers – who sometimes forget to talk to each other.**
To all of these challenges, DNA barcodes bring new solutions and new resolution. They allow us to get the nodes and the links right – and to store our data in a way that future ecologists can still use them. In both aquatic and terrestrial environments, molecular approaches to reconstruct nodes and the links of webs are exposing unexpected complexity: food webs are proving to be composed of more nodes linked in more ways than we previously knew. Molecularly resolved food webs allow for improved comparisons of whether webs in different environments are similar or different from each other, and to what extent they are linked by members passing from one environment to another. Finally, they allow us to link predator-prey interactions with other types of ecological interactions – like who pollinates whom or who disperses whom. These are exciting times for anyone interested in how nature is built and functions.

_Based on the following article:_
A study led by researchers from the University of Jordan and the Smithsonian Institution reports the first survey of the typically overlooked cryptic organisms found on reefs of the Jordanian coast of the Gulf of Aqaba (Northern Red Sea) using a standardized sampling protocol and high-throughput DNA barcoding (metabarcoding).

**Threatened Biodiversity**

The 27 km Jordanian coast of the Gulf of Aqaba, located in the Northern Red Sea, is fringed by highly diverse reefs that contain many endemic species. These most northerly reefs of the entire Indian Ocean may represent a refuge from the effects of global warming. However, the region suffers from severe anthropogenic impacts, resulting in pollution, habitat destruction, and overfishing. The resulting threats to the diversity of this unique reef ecosystem are yet to be fully characterized, making it imperative to conduct comprehensive biodiversity studies of this valuable natural asset.

**DNA Barcoding and Metabarcoding Tools for Marine Biodiversity Monitoring and Conservation**

Previous studies in the Gulf of Aqaba, a small semi-enclosed system in the Northern Red Sea, have primarily focussed on corals and fishes. Studies that considered other community members (i.e. sponges) were also based on classical survey methods that ignored the cryptic species hidden within the reef. This study addresses this knowledge gap by presenting the first comprehensive biodiversity estimates of the cryptic reef community based on DNA barcoding.

The project started in 2011 when Autonomous Reef Monitoring Structures (ARMS) were deployed among fringing reefs on the Jordanian coast. ARMS consist of a stack of PVC plates separated by spacers, and they can be deployed without destroying habitat. They represent a standardized sampling approach to characterizing communities of otherwise hard-to-survey cryptic organisms, both motile and sessile, occurring on reefs.
So much diversity in the Gulf of Aqaba remains unrepresented in DNA sequence databases, including the four most abundant crustaceans that we encountered. The ARMS were retrieved 18 months later; non-sessile organisms were sorted by size, and the sessile community was scraped off the plates. Non-sessile organisms >2 mm were individually DNA barcoded using standard protocols; this revealed 83 Operational Taxonomic Units (OTUs) in six phyla, of which only 25% matched a reference sequence in public databases. Not only is this a low percentage overall for these larger members of the cryptic community, but the sequences for the four most common species were not in the databases. For the smaller non-sessile fraction (2 mm-500 μm) and the sessile fraction, High-Throughput Sequencing (HTS) of the COI gene was used to generate metabarcoding data. This revealed vastly more diversity – 1197 OTUs in 15 animal phyla – of which only 4.9% matched reference barcodes.

These results highlight the scarcity of COI data for cryptobenthic organisms of the Red Sea.

Compared with data obtained using similar methods elsewhere, the results of this study suggested that Gulf of Aqaba reefs are less diverse than two Pacific coral reefs but much more diverse than an Atlantic oyster reef at a similar latitude. These results provide insights into regional-scale patterns of cryptobenthic diversity and highlight the potential of these methods for understanding diversity of the most overlooked reef organisms. HTS approaches show promise not only for establishing baseline data on biodiversity, but also for monitoring shifts in community structure due to global climate change and local anthropogenic stresses.

For more information about the results discussed in this article, see DOI: 10.1039/c0xx00000x.

Images by Matthieu Leray.
What is forensic palynology?

Imagine that you are a detective working on a missing person case. You have arrested a suspect, and you want to trace that suspect’s movements. A method that could be informative is forensic palynology, the application of palynology (the study of pollen) to forensics. Pollen is essentially ubiquitous in the environment, and the specific “signature” of which pollen grains are present somewhere is specific to particular places (because different plant species occur in different areas) and times (because those plant species flower at different times in different places). Pollen is also extremely durable. This all means that pollen is an ideal biomarker for linking people and objects to particular places and times, a central need in forensic investigations.

When mass graves were uncovered following the Bosnian war, it was suspected that bodies had been moved from different sites. Pollen was one of the lines of evidence used to trace these movements (Brown, 2006, DOI: 10.1016/j.forsciint.2006.05.025). There are many other cases where forensic palynology can be applied. For example, pollen grains that have settled inside an explosive device could be examined to determine where the device was assembled, or country of origin could be determined for imported products of questionable provenance.

How is forensic palynology currently applied?

Traditionally, pollen species identification has depended on visual examination under the microscope. Few people are trained in these methods; for example there is only one full-time forensic palynologist in the US. Some species have sufficiently unique pollen for species-level identifications with this method, but more commonly pollen is identified at the level of genus or even family. For these reasons, forensic palynology has been very underutilized. Recently, techniques have been developed to identify pollen using DNA barcoding. This has the potential to transform forensic palynology and allow us to harness the power of pollen to solve crimes (Bell et al., 2016, DOI: 10.1016/j.fsigen.2015.12.010).
How does DNA barcoding of pollen work?

DNA barcoding of pollen has three important prerequisites. The first of these is a set of genetic markers ("barcoding loci") that can be amplified across the seed plants and that can reliably distinguish between a high proportion of species. Second, because pollen samples contain a mixture of species, we need methods for simultaneously sequencing samples consisting of multiple species ("metabarcoding"). Finally, we need a database containing DNA sequences of the genetic markers for the majority of species of seed plants.

The first and third prerequisites are common to all DNA barcoding studies. The second prerequisite is unique to studies that involve species mixtures. Mixtures can be DNA barcoded through separating and sequencing the species individually, but this has the same inefficiencies as visual examination. Recent developments in high-throughput DNA sequencing make identification of mixtures more feasible. These methods allow multiple pieces of DNA to be sequenced at the same time, without separating them first. With high-throughput sequencing, the whole mixture of pollen grains can be ground up in the one sample, and the DNA of all species isolated and sequenced together. This technique is known as DNA metabarcoding.

Are we ready to start using these methods?

Pollen DNA barcoding has been successfully applied to quality testing of honey (Hawkins et al., 2015, DOI: 10.1371/journal.pone.0134735), identifying the plant species on which bees have been foraging (Richardson et al., 2015, DOI: 10.3732/apps.1500043), and air quality monitoring (Kraaijeveld et al., 2015, DOI: 10.1111/1755-0998.12288). Optimizing these methods for forensics may require some small modifications, such as dealing with very few pollen grains in a sample. Standardized methods should be developed to enable comparisons between different cases. It may also be necessary to expand the reference databases to include more species that might be of interest to forensics specialists. While there are still some challenges, DNA barcoding of pollen is poised to transform forensic science in the near future.
Species-based faunal monitoring is the most viable and reliable way to assess general ecosystem health. It contributes to early warning systems for potential pest gradations and allows for the early detection of invasive species, the study of spatial and temporal dynamics of species within an ecosystem, and the definition of areas for conservation priority settings, among other important predictions on biodiversity and ecosystem management.

Insects are the major component of biodiversity in virtually all terrestrial ecosystems, making them crucial for environmental impact assessment. In this context, Malaise traps (tent-like traps that intercept flying insects) represent a simple and effective sampling method, which is maybe the most common approach to inventory biodiversity worldwide (www.globalmalaise.org). However, for some hyperdiverse insect groups, in particular hymenopterans and dipterans, even experts will need several weeks to identify a Malaise trap sample just to family level. The use of next-generation sequencing (NGS) within the DNA barcoding framework might offer an alternative to generate more objective, globally accessible data, providing a promising tool to analyze extremely large amounts of specimens (such as a one-year Malaise trap sample with up to one million individuals) rapidly and economically.

Since 2009, two major barcoding initiatives have been coordinated and scientifically supported by researchers of the Bavarian State Collection of Zoology (ZSM, Munich, Germany): the Barcoding Fauna Bavarica project (BFB – www.faunabavarica.de) and the German Barcode of Life Project (GBOL2 – www.bolgermany.de). These projects generated a reference library, which is particularly comprehensive for Central Europe, especially Germany. The reference library contains more than 20,000 animal species thus far, with focal groups being Coleoptera, Hymenoptera, Diptera, and Lepidoptera.
In this study, we aimed to evaluate the coverage of the German DNA barcode reference library by using 120,000 DNA barcode sequences of species with a corresponding Barcode Index Number (BIN) in BOLD. We also tested the importance of a pre-sorting step into insect orders to yield better results. For that, in 2015, we pre-sorted one single Malaise trap sample (one week – including approximately 5,000 specimens) into the insect orders of interest and extracted each group separately. An aliquot of each DNA extract was combined to simulate a “non-sorted sample”. Each DNA extract was amplified with a set of four primers targeting the COI-5’ fragment, and PCR products were sequenced separately on an Illumina MiSeq platform.

We developed a workflow in order to associate the resulting sequence reads with species names or BINs, if no valid binomials were present within the database. We used the sequences and the corresponding percentages of identical sites in the clusters resulting from the use of the four primers in order to create a scoring matrix, which allowed an estimation of quality/reliability for each detected sequence cluster.

Of the 5,500 sequence clusters produced by that workflow, we were able to identify 390 high-quality BINs (with at least 97% sequence similarity for each primer) within that roughly pre-sorted Malaise trap sample. We compared that result with the simulated “non-sorted sample” (268 BINs), and we found a total of 69% shared high-quality BINs.

This study demonstrates an approximately 30% reduction in the detection of species from a non-sorted sample in comparison to a sample that undergoes a time-consuming sorting process. These results may be of interest in cases of limited time, funding, and personnel.

This year, we expanded our study by adding a size-sorting step to the order-sorting workflow to determine if the number of detected BINs could be further increased. For that purpose, all Diptera, Coleoptera, Lepidoptera, and Hymenoptera were sorted out from five Malaise trap samples, separating them into micro- and macro-fractions. Preliminary results suggest that size sorting can play an important role in some groups, indicating that “size does matter” after all.

For more information about the results discussed in this article, see DOI: 10.1371/journal.pone.0155497.

Images by Bruno Cancian de Araujo.
The ecosystem service of pollination by insects is essential to human food security and terrestrial biodiversity. Despite the importance of pollination to mankind, often little is known about the specific faunal and floral players in the countless relationships between animals and wild or crop plants. As part of a subproject of the German Barcode of Life (GBOL), we are studying, identifying, and evaluating pollinators and plant-pollinator networks in agricultural landscapes. This is done by means of DNA barcoding, relying on the reference database already established for the German fauna and by extending the database where needed.

Our studies focus on two important crops (apple and caraway) and on two insect groups (Hymenoptera and Diptera). Some species of these groups are known to be important pollinators, e.g. wild bees and hoverflies, but for many others we are still in the dark concerning their role as potential pollinators.

For each crop, we set up experimental fields, with plots accompanied by flower strips or without flower strips. Earlier studies have shown that flower strips can enhance insect biodiversity because they provide additional food resources. Hence, the presence of flower strips will allow us to attract as many potential pollinators as possible and to monitor their presence and feeding beyond the flowering season of the respective crop.

In defined collecting schemes, both target crop and flower strips are searched for Hymenoptera and Diptera. Each specimen and its pollen load is individually stored. The insects are barcoded using standard procedures of GBOL; the pollen will be barcoded within a collaborative GBOL project that develops procedures for DNA-based pollen identification. With the detailed information on the pollen load of each potential pollinator, we will be able to draw plant-pollinator networks over the course of the year.

“With the detailed information on the pollen load of each potential pollinator, we will be able to draw plant-pollinator networks over the course of the year.”
Based on these networks, we will be able to answer the following questions: Which species are potential pollinators of the respective crop? When are they present? What else do they feed on over the course of the year?

With this background of species-specific knowledge, the complete barcode library of pollinators and pollen will enhance the development of measures to promote and conserve insect-plant networks in agricultural habitats, by providing the possibility of easy and fast identification of species. In the long term, this knowledge and infrastructure will be used to give recommendations to farmers so that they may foster their pollinator fauna in order to safeguard and improve local biodiversity and crop yield.

“...this knowledge and infrastructure will be used to give recommendations to farmers so that they may foster their pollinator fauna...”
Since 2009, the Museo Argentino de Ciencias Naturales (MACN) has hosted the Leading Labs Training Workshop for DNA Barcoding in Buenos Aires. With the explicit goal of “extending and enhancing DNA barcoding research in Argentina and neighboring countries”, this course/workshop has been attended by 60-90 researchers, students (mostly graduate), postdocs, and technicians from Argentina and other South American countries over the last eight years. The workshop is a joint initiative of MACN and the Centre for Biodiversity Genomics (CBG, University of Guelph). In its first four editions, researchers and graduate students from both MACN and CBG acted as instructors, but since 2013, Argentine instructors (mostly from MACN) have covered all aspects of the course.

The workshop is targeted mainly towards the recipients of the funding granted every year by the Argentine National Research Council (CONICET) for DNA barcoding (other agencies and foundations have also funded barcoding activities in Argentina, including Fundación Williams, the Richard Lounsbery Foundation, and Canada’s International Development Research Centre). The training consists of two different approaches. First-time attendees undertake a course with a mix of theoretical and hands-on activities covering all aspects of the barcoding pipeline, including general introductions, specimen processing, imaging and tissue sampling, laboratory work, and data analysis using BOLD and other platforms.

Current barcoding projects carried out in South America are also presented every year, providing insights into the state of the initiative in the region. Attendees are then invited to return to the workshop in the following years in order to process their own samples, which enables them to receive deeper training while also advancing their own barcoding projects.
The latest workshop took place this year between May 30 and June 3. As a result of this workshop, first-time attendees were introduced to DNA barcoding and its methodology while returning participants brought and processed a total of 1,870 specimens or samples, including material from Argentina, Uruguay, and Ecuador. The DNA barcodes of these materials are currently being obtained at the MACN barcoding molecular laboratory. Along with the concepts usually taught at the workshop, this year included the novelty of having a live presentation given by Megan Milton via Skype from CBG. Megan is the Project Manager of the Barcode of Life Data Systems (BOLD), and she introduced the audience to the new features of BOLD 4.0.

In addition to Argentine participants, 11 Latin American researchers attended the workshop this year, including representatives from Uruguay, Peru, Ecuador, and Colombia. In fact, Mailyn González, from the Conservation Genetics Laboratory at the Humboldt Institution in Bogotá, gave one of the presentations on the last day about ongoing projects in the region. She talked about the Colombian Barcoding Network and one of their projects related to the use of DNA barcodes to identify CITES-listed, endangered bird species and study their diversification history.

The next workshop is expected to take place at MACN in mid-2017. We expect that participants will come as eager to learn, process their materials, and interact with the rest of the regional barcoding community as they always do.
In the past hundred years, freshwater ecosystems have been dramatically altered and degraded by human activities worldwide. The massive impacts of stressors have led to a serious loss of biodiversity entailing a decline in resilience in these ecosystems. A main concern of such long-term impacts on freshwater ecosystems is the loss of central ecosystem functions, e.g., filtration, nutrient and carbon cycling, and production of biomass, with direct negative effects on people. Therefore, restoration and maintenance of these ecosystems is of significant interest, and comprehensive management and restoration programs have been launched in the last few decades (EU Water Framework Directives, WFD, Directive 2000/XX/EG; US Clean Water Act, 1972 and following; and Australian National Water Quality Management Strategy, NWQMS).

In order to make valid assumptions about ecological status and restoration success, extensive monitoring is necessary, which requires clear guidelines and standardized methods. Diversity and abundance of macrozoobenthic organisms are widely used for environmental impact assessments. A central problem is, however, that identification of these organisms to genus or species level is difficult and often gives unsatisfactory results. In addition, traditional determination is time consuming, and few experts exist for several taxonomic groups. Here, DNA metabarcoding can be regarded as an opportunity, but, even though it has been proposed in several studies for biodiversity assessments, the method is lacking standardized sampling procedures, laboratory processes, and bioinformatic pipelines.

"...extensive monitoring is necessary, which requires clear guidelines and standardized methods."
In our GBOL II subproject, we want to evaluate and standardize several steps in the process of metabarcoding, such as sample preparation and the bioinformatic pipeline for data analyses. Two very different stream ecosystems will be assessed: 1) the river Emscher in the Metropolis Ruhr, which is probably one of the most degraded river ecosystems worldwide; and 2) the river Sieg, which is a prototype of a healthy low-mountain stream.

The Emscher has been used as an open sewage channel for more than a hundred years, and large parts are still artificial and biologically almost ‘dead’ with respect to higher metazoan life. For the last several years, however, this stream has been the subject of one of the biggest and most expensive restoration projects worldwide, with a total budget of about €4.6 billion. The river Sieg is completely different with respect to anthropogenic alterations and is largely in near-natural condition.

As part of a PhD project, we will apply the currently developed and improved metabarcoding methods of our lab, e.g. the application of newly generated primers (see Elbrecht and Leese, 2016, DOI: 10.7287/peerj.preprints.2044v2) to real-world samples. Furthermore, the impact of the “organic pollution” stressor on macrozoobenthic communities will be analyzed, and the development of genetic diversity within these communities will be surveyed at certain restored sites. Together with data generated during the GBOL I phase, the river Sieg will be used to observe the influence of water level changes on macrozoobenthic communities over a period of three to four years. In collaboration with other projects, eDNA and metagenomic approaches will be studied as potential complementing techniques. Therefore, as part of the GBOL II project, we will compare these three innovative methods with respect to their applicability for biodiversity assessment.

The project also nicely connects to the recently granted EU COST Action DNAqua-Net (www.DNAqua.net) by linking research to practice even beyond the goals of GBOL II. We would be happy to engage in an active exchange of ideas and experiences with other working groups dealing with DNA-based water quality assessments.
“From DNA barcodes to biomes”, a special issue of the *Philosophical Transactions of the Royal Society*, is now available with full open access. Compiled and edited by Paul D. N. Hebert, Mehrdad Hajibabaei, and Peter M. Hollingsworth, the special issue features contributions derived from presentations at the 6th International Barcode of Life Conference.

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The project SPHINX, further titled ‘Understanding and predicting species adaptation to environmental changes in insects’, has been funded by the French National Research Agency (ANR) in the amount of €495K over four years. The project will begin in January 2017 and will build on distribution and trait databases, along with the macroecology study carried out as part of project ACTIAS (funded by CESAB; see Barcode Bulletin vol. 6(2), June 2015).

SPHINX will target two diverse families of moths, Saturniidae and Sphingidae (ca. 4,500 species in total). The goals of SPHINX are:

(i) to build a comprehensive species-level phylogeny and to conduct the first diversification analysis of a diverse group of insects on a global scale incorporating the role of biotic (e.g. dispersal ability and diversity of host plants) and abiotic (e.g. climatic and geological changes) factors. This phylogeny will benefit from the nearly comprehensive DNA barcode libraries for the two moth families and will combine a set of phylogenomic approaches (RADSeq, gene capture, and mitogenomics);

(ii) to analyse the evolutionary dynamics of ecological niches and to develop macro-evolutionary models combining phylogenetic, biogeographic, and ecological variables; and

(iii) to experimentally test the ability of these models to predict species responses to environmental changes by analysing, in the field, species communities of these moths in pristine and human-impacted habitats on three different continents.