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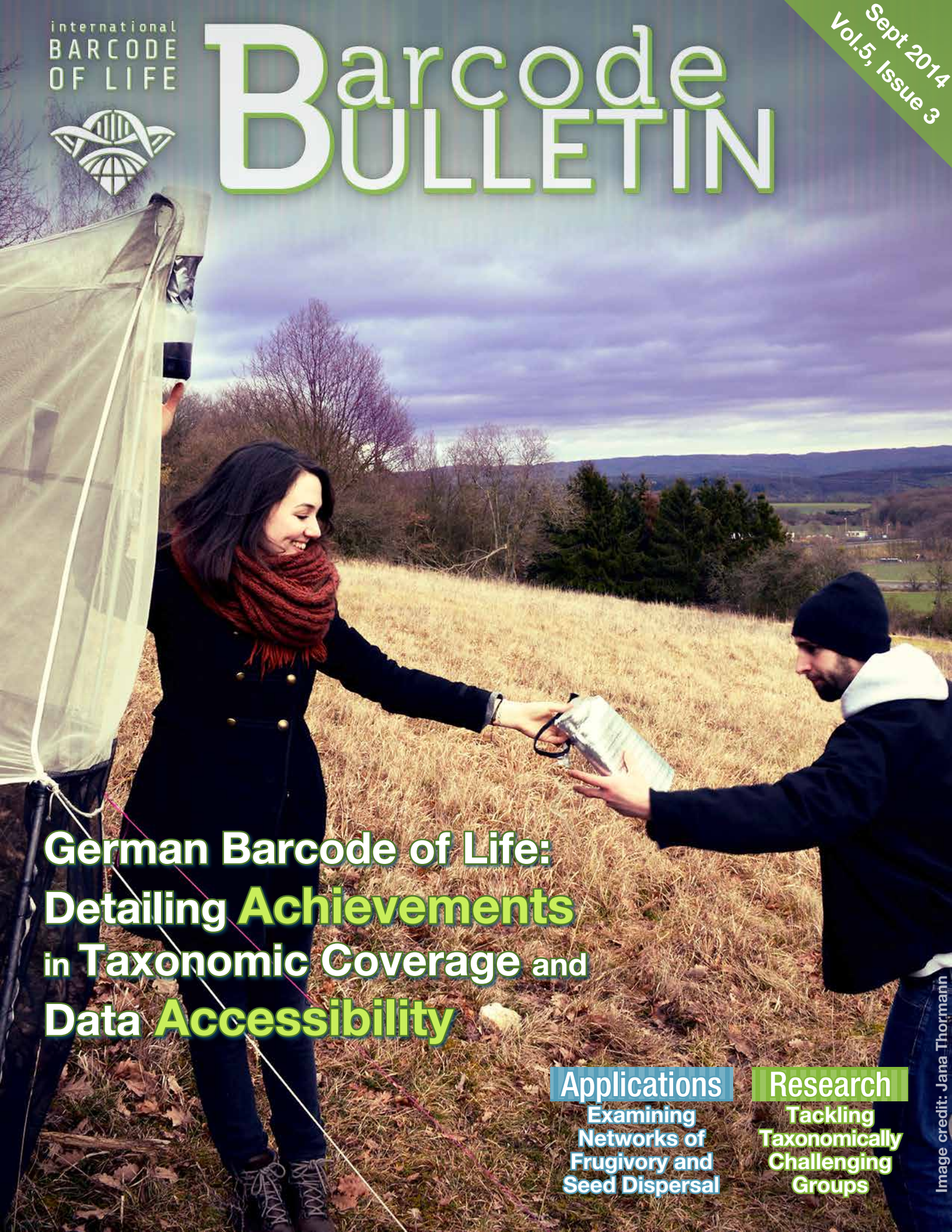

German Barcode of Life: Detailing Achievements in Taxonomic Coverage and Data Accessibility

Applications
Examining Networks of Frugivory and Seed Dispersal

Research
Tackling Taxonomically Challenging Groups

Image credit: Jana Thormann

Welcome to the September 2014 issue of the iBOL Barcode Bulletin.

This is the second issue with a new format. We received some very positive comments about the changes made to the last issue which encouraged us to keep going in this direction. Currently we are working on the development of a tablet version of the Barcode Bulletin which will be released with the December issue or with the first issue in 2015.

This issue contains nine articles from seven countries spanning three continents demonstrating how far the DNA barcoding enterprise has spread. We are glad to be able to showcase advances in technology and application.

Enjoy reading.

Dirk Steinke
Editor-in-chief

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News Briefs

The National Museum of Natural History in Paris, France has appointed Rodolphe Rougerie as Assistant Professor and Curator of the Rhopalocera section of their Lepidoptera collection. The museum is a public institution for research and education in Earth and Life Sciences; it hosts some of the world's largest and richest natural history collections, with estimates of 68M specimens and 800K types. The Lepidoptera collection comprises about 4M pinned specimens, of which 1/3 are butterflies. In addition to his curatorial role, Dr. Rougerie will conduct research on the systematics, evolutionary history, biogeography, and macroecology of Lepidoptera. He will specifically look at the role of key adaptations in the diversification of these insects, with a focus on bombycoid moths and in particular members of the families Sphingidae and Saturniidae.

A China National Science Foundation grant of 840K RMB has been awarded to a group of researchers, which includes Laurence Packer and Jason Gibbs, to barcode the sweat bee genus *Lasioglossum* in China. The project will run from January 2015 to December 2018.

The San Diego Biodiversity Project has been awarded $300,000 by the National Science Foundation to collaborate with BIO and community colleges in southern California on a regional barcoding effort. Palomar College, Mt San Jacinto College, and Cuyamaca College will be integrating DNA barcoding into their biology curriculum. Along with UC San Diego, undergraduate students will be collecting insects and other invertebrates from their campus and environs for DNA barcoding in their classroom laboratories. BIO will be creating a college version of the Student Data Portal, so students from different campuses can collaborate, compare data, and ultimately upload their data to BOLD. The goal of the project is to increase undergraduate student engagement and retention in science by giving them the opportunity to contribute to original research. Any 2 or 4-year colleges interested in getting involved should contact Heather Henter, hhenter@ucsd.edu.

Feature

Image credit: Stephanie Pietsch

German Barcode of Life: Laying Foundation for Future Applications

Written by: Matthias Geiger on behalf of the GBOL team

Since November 2011, the German Ministry of Education and Science (BMBF) has been providing substantial funding (ca. 5 Million Euros) to a national consortium of 17 natural history museums and biodiversity research institutions. The ultimate goal is to construct a DNA barcode reference library for the fauna, flora and fungi of Germany.

About 50 GBOL institution-based and over 200 citizen scientists provide their professional taxonomic expertise and existing infrastructure (e.g. dry and wet collections/biobanks, databases, bioinformatics platforms and laboratories). The Zoological Research Museum Alexander Koenig (ZFMK) in Bonn and the Bavarian State Collection Munich (ZSM) coordinate this campaign, and a national web portal for DNA barcodes and associated specimen data (www.bolgermany.de) has been developed.

"Thus far, DNA barcodes for about 15,000 animal species have been generated."

As a starting point for the project, species numbers for Germany were taken from a review provided by the Federal Agency for Nature Conservation in 2009, which estimates that 48,000 animal and 10,000 plant species (excluding algae and fungi) are present in Germany.

The goal for the first phase of GBOL (2011-2015) is to generate DNA barcodes for over 20,000 species of animals, plants and a few selected rust fungi (Pucciniales) with up to 10 individuals per species from several locations throughout Germany.

All DNA extracts are stored in the DNA Bank Network (www.dnabank-network.org). To tackle the high diversity of taxonomic groups covered, the processing strategy consisted of assigning work-packages to research institutes with expertise in a specific area:

GBOL-1: ZFMK for selected animal taxa

GBOL-2: ZSM for selected animal taxa

GBOL-3: State Museum of Natural History Stuttgart, State Museum of Natural History Karlsruhe, Julius Kühn-Institut for selected animal taxa and fungi

GBOL-4: Senckenberg Museum for Natural History, University Bielefeld for soil fauna

GBOL-5: Nees Institute for Plant Biodiversity, Berlin-Dahlem Botanical Garden and Botanical Museum, State Museum of Natural History Stuttgart, Institute for Evolution and Biodiversity Münster, Botanical State Collection Munich for the Flora

Different strategies have been pursued to accumulate the data, which are currently being assembled to make them available to users via a single national platform. ZFMK has developed a sample and data processing scheme which includes a field information management system (FIMS) (represented by the Diversity Workbench Framework http://diversityworkbench.net/) and automatic data exchange/annotation with the laboratory information management system Geneious Pro). All specimen and collection-related information along with the sequencing data are transferred to, and displayed on, the continuously enhanced web portal www.bolgermany.de.

ZSM and some other institutes already had pre-GBOL projects or partnerships with the Canadian Centre for DNA Barcoding, with direct dataflow to and from the Barcode of Life Data Systems (BOLD). The GBOL-2 sub-project of the ZSM has profited from an excellent network of professional and amateur scientists engaged since 2009 in the "Barcoding Fauna Bavarica" project which was financed by the Bavarian State Government. Together with the BOLD team, GBOL is currently adapting the data exchange with the BOLD Web Service Interface to be compliant with the international ABCDDNA standard.

"...focus more strongly on establishing DNA barcoding as a tool for practical applications."

Unusual Malaise trap finding: the wingless snow fly *Chionea belgica* (Becker, 1912), first record for the state of Rheinland Pfalz in SW Germany.

Image credit: Björn Rulik

Thus far, DNA barcodes for about 15,000 animal species have been generated and we are confident that the project's goal will be reached by April 2015. A feature of the GBOL Botany section is to provide sequence data for the approximately 4,800 native land plant species of the German flora not only for the official barcode markers rbcL and matK, but also to complement them by lineage-specific fast evolving plastid DNA regions (trnL-F, rpl16, trnK/matK) in combination with the nuclear internal transcribed spacers (ITS) to achieve higher resolution. GBOL expects an increased demand in the future, especially for the nrDNA ITS marker, for example in applications that involve the analysis of pollen.

Future efforts of the GBOL consortium in an anticipated second funding period will focus more strongly on establishing DNA barcoding as a tool for practical applications. Important steps towards this goal are ongoing pilot studies on the impact of climate change and forestry management practices on biodiversity. Examples include ZSM's participation in the Global Malaise Trap Program, with traps in Bayerischer Wald National Park and in an altitudinal transect in the Alps, as well as ZFMK's cooperation with Eifel National Park to analyze mass samples from pitfall and Malaise traps and soil probes.

Along with the increasing demand for practical applications, we are well aware that, in the end, only reliable and comprehensive reference libraries as a baseline will enable us to fulfill the promises of DNA barcoding.

Collecting different meat samples for DNA barcoding.

A Challenging Experiment for the Average Newbie

Written by and photos by: Claudia Marginean (Waag Open Wetlab, Amsterdam)

In the centre of Amsterdam, flanked by the culinary delights of Chinatown and the temptations of the Red Light District, stands the Waag building. In this building, you can find the Open Wetlab - a place for the delights of science and temptations of the mind! Anyone is welcome here, but you are advised to bring two things. First, a curious mind. Second, a desire to experiment with biology. Whether you experiment for fun, for art, for science, or even to explore modern ethical boundaries is entirely up to you. The only requirement of Do-It-Together Biology at the Waag is that you must share your work online with everyone else.

Bring two things: curiosity, and a desire to experiment with biology.

In June, I decided to start an experiment suitable for the average newbie. An experiment that would be simple enough not to require a biosafety permit, but that would also be challenging and informative. I selected the 'Mystery Meat experiment', using a protocol written by Manchester Madlab. This experiment is based on a simplified version of DNA barcoding and teaches some of the most common and useful concepts in molecular biology, including DNA extraction, polymerase chain reaction (PCR), and gel electrophoresis.

It was a different thing altogether to look at almost empty tubes, while knowing they actually contained DNA.

The Waag Wetlab had everything I needed, except for the primers for the meat. I ordered DNA primers online that would identify proteins for chicken, beef, pork, horse, goat and sheep. They arrived two days later, a small selection of unassuming, translucent vials. It made me realise something anew. DNA is very small and cannot be seen based on size or colour. While I had known this before, it was a different thing altogether for my eyes to tell me these tubes looked empty, while my brain assured me they really did contain DNA.

But before I could go uncovering another horsemeat scandal, I needed to start with the basics. I needed to be able to show distinct DNA barcode profiles for the different meats. Our lab group collected small pieces of meat of chicken, fish, beef and pork from ordinary lunch foodstuffs. We extracted DNA from the samples, added a blend of the DNA primers, added PCR mix, and then ran each solution through the PCR process. In each meat sample, only a specific DNA fragment would be amplified... At least, that was what was supposed to happen. Instead, something went wrong. After the PCR process had finished, Pieter, our resident lab manager, noted that the tubes contained less solution than expected. And when I used gel electrophoresis to see what DNA had been amplified in each sample, nothing could be seen.

The mystery could only be untangled by trying the experiment again. So I ran a second attempt, which I called a 'debugging' experiment. For simplicity I only used beef and pork samples. I paid extra close attention to solution quantities, and I used a different kind of PCR mix for half of the tubes.

"The mystery could only be untangled by trying the experiment again."

This time, after gel electrophoresis I could see a couple of bands of DNA from the tubes that contained the original PCR mix. So the mix was not the problem. Then why didn't I see DNA in the first round of the experiment? I'm not sure but I will find out.

Unfortunately, the differences between the pork and beef DNA profiles were still not conclusive. So I've now started a third run of the experiment, and this time, besides pork and beef, I am testing vegetarian meat, which shouldn't show any bands on the gel, should it? We'll see...

Many thanks to Pieter, Julia and Anna for their assistance with this experiment.

The Waag Open Wetlab cupboard.

Preparing to run gel electrophoresis for the first attempt.

Results of the second attempt... DNA is present, but profiles are still inconclusive!

Commercial fish from an Egyptian market. Image credit: Asmaa Galal-Khallaf

DNA Barcoding for Authentication of Egyptian Fish Fillets

Written by: Asmaa Galal-Khallaf, Khaled Mohammed-Geba (Menoufia University, Egypt), and Eva Garcia-Vazquez (University of Oviedo, Spain)

Egypt is home to some of the most popular fish in the world and is the top African country in aquaculture production. The Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1758), which is cultured in ponds and floating cages in all tropical and subtropical areas from the Americas to China, accounts for more than 50% of total aquaculture production in Egypt. Besides large-scale aquaculture for international trade, it is also cultured on a subsistence level to meet local protein needs. Its local popularity as a resource is not new. The species has been cultivated in Egypt since the beginning of written history; back to 2,500 B.C., where tombs illustrate the harvest of Tilapia from artificial ponds.

Another popular African fish is the Nile perch (*Lates niloticus*, Linnaeus 1758), a delicacy with firm flesh and delicious taste that is mainly obtained from extractive fisheries particularly from Lake Nasser in Upper Egypt, with an annual catch of more than 300,000 tons. While Nile perch aquaculture started in the late 1990s, it currently produces less than 20,000 tons annually.

"...fraud due to species substitution has been reported worldwide."

Recently, other species have joined the Olympus of fish popularity in Egypt. One example is the imported Vietnamese Basa fish (*Pangasius bocourti*, Sauvage 1880), which has white flesh and is highly valued. Its sibling, Tra fish (*Pangasionodon hypophthalmus*, Sauvage 1878), is another Vietnamese catfish intensely commercialized in the country although at a lower price due to doubts about its quality.

Fish fillets are considered convenience food. They can be stored frozen for a long time and do not require any processing like eviscerating and cleaning before cooking as needed for whole fishes. However, the consumer cannot recognize the fish from the fillets, and fraud due to species substitution (generally the cheaper for the more expensive) has been reported worldwide.

This very first study of Egyptian fish markets started in 2013. We purchased fish fillets of different commercial brands from fish markets of the Egyptian governorates of Monufia, Cairo and Qalyubia (N=90). DNA extraction and PCR amplification

of the cytochrome oxidase subunit 1 gene were 100% successful. Comparing the obtained COI sequences with reference sequences on GenBank and BOLD, we were able to identify all of the sampled fish fillets to species. Moreover, the sequences exhibited clear phylogenetic signal that enabled species assignment by Neighbor-Joining phylogeny reconstruction.

The results were surprising. On one hand all fillets sold as Nile Tilapia were correctly labeled, but half of the fillets labeled as Basa were really Tra catfish. Moreover, and hardly believable because of the substantial difference in flesh quality, half of the Nile perch fillets were also Tra catfish.

"Up to 50% of the highly appreciated Nile perch fillets sampled from Egyptian markets were really Tra catfish."

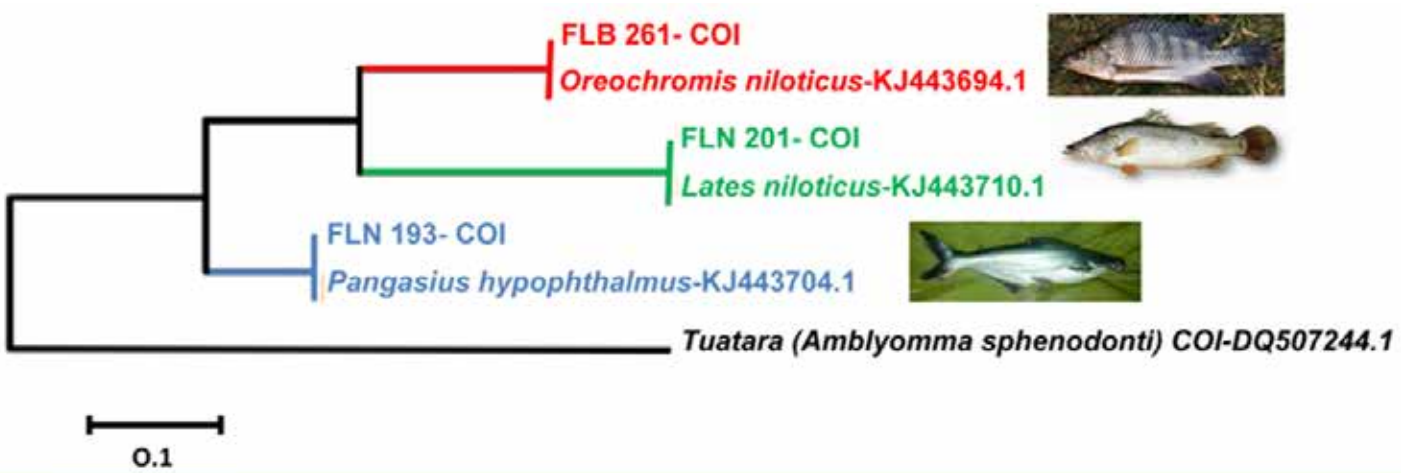
The only one of these species to occur on IUCN red-lists is the Tra catfish. This species is listed as endangered but the substitute found in this study is most likely an aquaculture product, therefore this fraud has no apparent consequences for ecosystem health.

However it raises concerns for those consumers allergic to panga catfish that buy what they believe is Nile perch but is really Tra catfish. Indeed it is also economically unfavorable for all consumers: they pay for a quality species and get a cheaper one instead.

We propose the use of barcoding for routine monitoring of commercial fish in Egyptian markets. This will help authorities in controlling and ensuring label authenticity thereby providing assurance to local consumers.

This is a contribution from the Marine Observatory of Asturias.

For more information about the results discussed in this article, see DOI: 10.1016/j.foodcont.2014.06.016



Phylogenetic tree reconstructed from reference COI Barcodes and COI sequences obtained from commercial fillets. Image credit: Asmaa Galal-Khallaf. Fish photos were taken from: Perch, www.Globefish.org; Tra, www.fishbase.org; Tilapia, www.zipcodezoo.com.

Image credit: Miles Zhang

Integrative Approach to Species Delimitation in Eurytomidae

Written by: Miles Zhang (University of Manitoba)

One of the most specialized forms of plant feeding among the insects is the ability to induce galls, which are highly-regulated growths on plants that serve as a source of high-quality food and shelter from adverse weather. These conspicuous bundles of food are often susceptible to attack by various species of small wasps known as parasitoids; the ones that feed on the inducers are referred to as parasitoids, and those that feed on gall tissues are referred to as inquilines.

The gall wasps in the family Cynipidae are capable of inducing some of the most structurally complex galls, and one group that is responsible for galls commonly observed on wild roses is *Diplolepis*. Parasitoids in the family Eurytomidae are among the most common parasitoids of *Diplolepis*, often comprising about 40% of total emergents.

Combining morphological, molecular, and ecological data to investigate the complex relationships between eurytomids and their hosts.

Ten species of eurytomids are known to be associated with galls of *Diplolepis* in Canada, feeding on either the inducer or inquilines. However, they are extremely difficult to identify using morphology alone, and thus a novel approach is needed to delimit these morphologically similar species.

With the advances in molecular biology, the use of molecular markers has proven essential for delimiting closely-related species among hymenopteran parasitoids. COI has been shown to be a valuable tool in identifying cryptic taxa, in combination with morphological and ecological data, for testing host-specificity and geographical variability for Hymenoptera, including members of Eurytomidae. The aim of this study was to use morphological, molecular, and ecological data to delimit these species.

A total of 423 eurytomids reared from the galls of all 14 species of *Diplolepis* found in Canada were barcoded in our study, using a combination of primers due to the difficulties in amplifying chalcidoid mitochondrial DNA. Eight morphospecies of eurytomids were found in association with galls of *Diplolepis*, including five of the ten species previously known from Canada. With the exception of *Tenuipetiolus ruber*, the seven other species belong to the large genus *Eurytoma* within the *E. rosae* species group. Incongruences were found between the resulting haplogroups and existing species. The presence of a previously overlooked cryptic species, "*E. spongiosa* 2" was found (to be described in an upcoming publication), and three species were shown to be just one morphologically variable species through molecular data.

In addition, DNA barcoding allowed us to associate the previously unidentifiable male specimens with their female counterparts, thus discovering new morphological characters on the antennae and petiole that are useful in distinguishing the male specimens. Perhaps most interestingly, the host range of many of the *Eurytoma* species were discovered to be broader than previously thought, including bivoltine species such as *Eurytoma longavena* where the first generation attacks *Diplolepis polita* in the spring while the second generation attacks and overwinters in a later summer gall of *Diplolepis nebulosa*.

The presence of synonymous and cryptic species in this study has highlighted the need for molecular data in combination with other datasets in the species identification of Eurytomidae. The taxonomy of Eurytomidae is in need of revision, and species in the large genus *Eurytoma* are particularly problematic due to their small size and broad host range. By incorporating molecular data with morphology and host data, the complex relationships between these taxonomically challenging eurytomids and their hosts can be slowly teased apart.

For more information about the results discussed in this article, see DOI: 10.4039/tce.2013.70

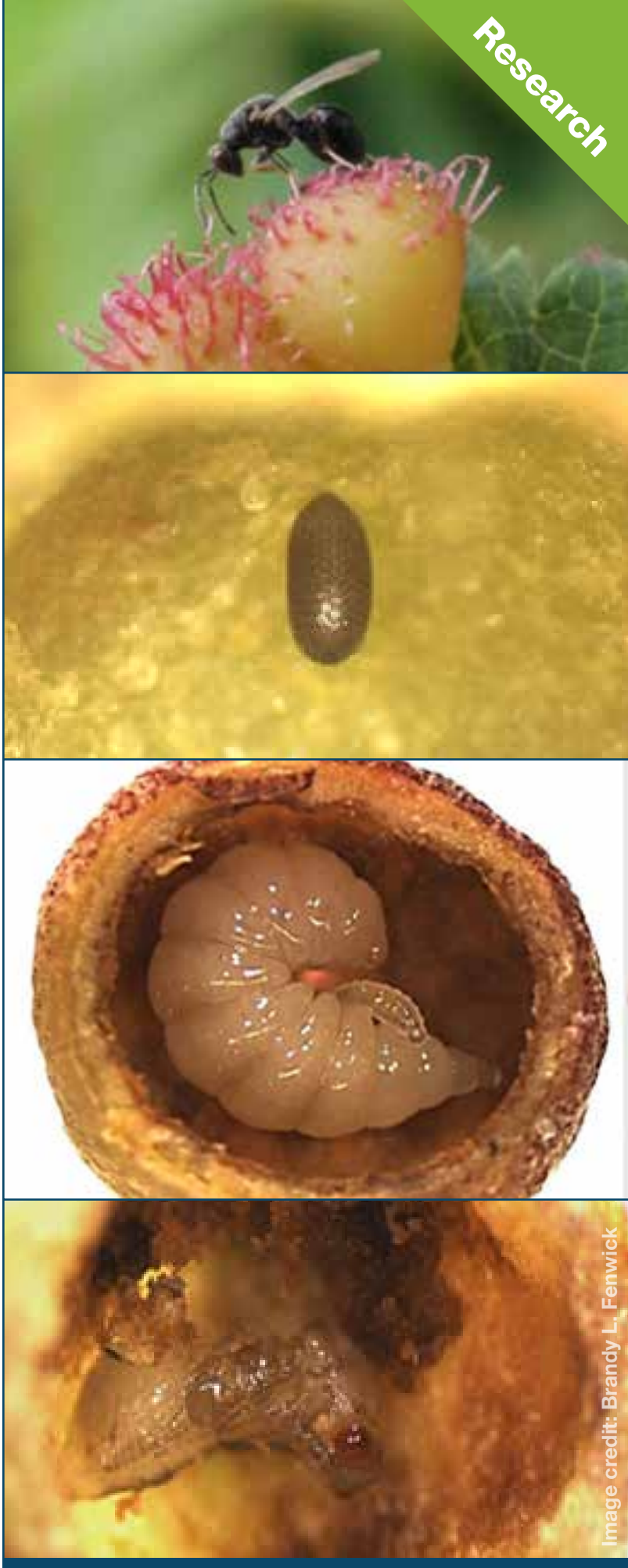


Image credit: Brandy L. Fenwick

Typical life cycle of a eurytomid (top to bottom): oviposition, egg, larva feeding on host, pupa.

Image credit: Juan Pedro González-Varo

Finding the Bird that Dispersed the Seeds

Written by: Juan P. González-Varo, Juan M. Arroyo, and Pedro Jordano (Estación Biológica de Doñana, EBD-CSIC, Spain)

Seed dispersal by vertebrate frugivores is a mutualistic interaction in which the animals benefit from eating the edible pulp of the fleshy fruits while the plants benefit from the movement of their seeds. Frugivores ingest fruits, transport seeds in their guts, and regurgitate or defecate them in conditions that may be suitable for germination.

This mutualism involves, for example, up to 98% of tropical rainforest or 60% of temperate woody plant species. Understanding the dispersal vector species for different plant species is crucial to assess the consequences of species extinctions and to foresee the potential collapse of forest regeneration due to loss of key dispersal services.

A methodological issue that has limited advances in our understanding of seed dissemination by frugivorous animals and its consequences is identifying the frugivore species that dispersed the seeds sampled in field studies.

On one hand, obtaining direct observations of frugivores feeding on fruits and using different landscape sectors is often complicated owing to the elusive character of the species or to the presence of dense vegetation. On the other hand, visually identifying frugivore species from faecal remains is impossible whenever they belong to closely related or similar-sized species.

"Now, DNA barcoding opens new research avenues for frugivory and seed dispersal studies..."

Specific identification is a crucial aspect to determine which frugivore species contribute to critical dispersal events (e.g., long-distance dispersal) that allow the connectivity of plant populations and the colonization of non-occupied habitats. Ultimately, specific identification is necessary to understand the particular role of different frugivores in the dynamics and regeneration of the vegetation.

We have described DNA barcoding protocols (GuSCN-silica based, for degraded DNA) successfully applied to bird-dispersed seeds sampled in the field. During the winter of 2013–2014, we periodically sampled seeds of fleshy-fruited plants in seed traps that were placed across different microhabitats of Mediterranean woodland. Avian DNA was extracted from the surface of defecated or regurgitated seeds (i.e., remains of avian digestive tissue), allowing the identification of the frugivore species that contributed each seed dispersal event. Disperser species identification was based on a 464-bp mitochondrial DNA region (COI: cytochrome c oxidase subunit I). The obtained sequences were identified using the BOLD identification engine.

Now, DNA barcoding opens new research avenues for frugivory and seed dispersal studies as it provides a non-invasive technique that allows quantifying frugivory and seed dispersal mutualistic networks, assessing the contribution of each frugivore species to seed rain in different microhabitats, and testing whether different frugivore species select different fruit/seed sizes. DNA barcoding is thus applied to identify interactions, rather than species.

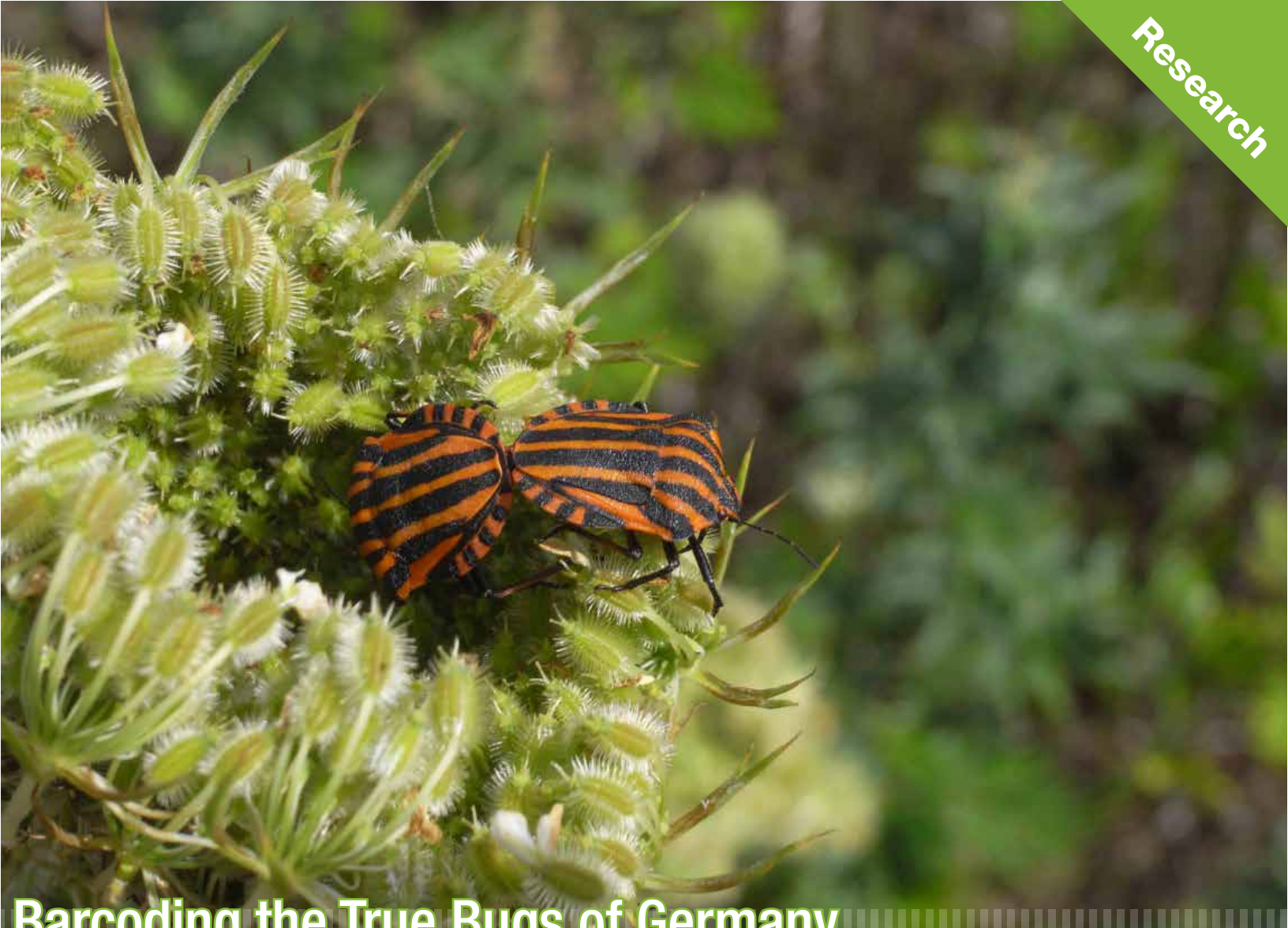
Given that seeds are sampled at the end of the dispersal process, this method enables linking the identity of the disperser species responsible for each dispersal event to plant traits (e.g. fruit/seed size) and environmental features (e.g. habitat/microhabitat of destination). It is precisely this bridge between frugivory and seed deposition patterns at the individual seed level which was unavailable to traditional field studies.

In conclusion, DNA barcoding can be used for characterizing the functional value of specific frugivore species within diverse mutualistic assemblages, opening new avenues to identify critically central interactions sustaining the web of mutual interdependencies between animals and plants.

For more information about the results discussed in this article, see DOI: 10.1111/2041-210X.12212

The edible pulp of fleshy-fruits is a food source for frugivores. The seeds are then transported in their gut and deposited via defecation or regurgitation.

Bird-dispersed seeds were sampled in seed traps placed in different microhabitats.



Barcoding the True Bugs of Germany

Written by: Michael J. Raupach (Senckenberg) and Martin M. Gossner (TU München) Photos by: Martin M. Gossner

True bugs or Heteroptera represent a highly diverse taxon of the Hemiptera which count as one of the big five insect orders in terms of species richness. Until now, more than 42,000 species have been described worldwide, with about 900 species known from Germany.

Whereas most species are terrestrial, a few are aquatic. Some species which feed on plant juices are infamous pests of cultivated crops (e.g. species of the genus *Lygus*), whereas other species are predacious and benefit man as effective biocontrol agents. Some parasitic species are also known as carriers of disease.

"...emphasizes the utility of DNA barcodes for the identification of true bugs..."

Due to their high ecological and economic importance a reliable species determination of true bugs is highly desirable. However, the occurrence of a high number of morphological similar species, e.g. in several genera of the Miridae, the by far most species rich family, makes a morphological identification difficult and time consuming. Furthermore, the identification of nymphal stages or eggs is even more critical or even impossible, although necessary, e.g. for early stage detection of potential pest species. between environment and space.

"...1700 specimens of 457 species collected in Germany, representing the largest DNA barcode library for true bugs so far."

In contrast to various other insect taxa, barcoding analyses of true bugs are still limited to some pioneering studies. Our study, just published in PLOS ONE, comprises DNA barcodes for more than 1700 specimens of 457 species collected in Germany, representing the largest DNA barcode library for true bugs so far. Using BOLD, low nucleotide distances with a minimum pairwise K2P distance < 2.2% were found within 21 species pairs (39 species). For ten of these species pairs (18 species), minimum pairwise distances were zero. In contrast to this, deep intraspecific sequence divergences with maximum pairwise distances > 2.2% were detected for 16 traditionally recognized and valid species.

With a successful identification rate of 91.5% (418 species), the present study emphasizes the utility of DNA barcodes for the identification of true bugs and represents an important step for building up a comprehensive barcode library for true bugs in Germany and Central Europe as well. However, the study also highlights the urgent necessity of taxonomic revisions for various taxa of the Heteroptera, with a special focus on various species of the Miridae, even for Europe. Consequently, the collaboration of morphological and molecular taxonomists will be crucial for resolving the uncertainties in Heteroptera taxonomy.

For more information about the results discussed in this article, see DOI: 10.1371/journal.pone.0106940

Subterranean *Lasius* ants tending *Prociphilus* aphids. Image credit: Daniel Kronauer

Unravelling a Mutualistic Ant-Homopteran-Microbe Network

Written by: Aniek Ivens (The Rockefeller University)

Mutualism, cooperation between different species, is widespread throughout nature. From plant-pollinator interactions to the cooperation between ourselves and our gut bacteria: beneficial interactions between species are everywhere and very important for everyday life.

Nevertheless, the evolution of mutualism remains poorly understood. How do species evolve to work together when as parasites they could reap the benefits without paying the costs? By comparing different mutualisms to each other, scientists can identify universal mechanisms that allow mutualisms to persist during millions of years. Specificity likely is such a feature. By specializing on a single partner, mutualists become dependent on each other for survival, thus preventing each other from evolving into parasites.

Mutualism is everywhere, yet its evolution remains poorly understood.

Interactions between ants and homopterans (e.g. aphids, mealybugs) are good examples of such ancient mutualisms. The ants milk the homopterans for 'honeydew' (their sugary excrement) while the homopterans feed on plant sap in a predator-free and clean environment provided by the ants.

These nutritional mutualisms do, however, not only involve insects. Also mutualistic bacteria likely play important roles: aphids harbor a specialized bacterium, *Buchnera*, which provides essential amino acids to its hosts. *Buchnera* thus enables aphids to feed on nutrient poor plant sap, ultimately resulting in honeydew. Mealybugs also have such 'endosymbionts'. And in turn the ants' ability to use honeydew as their main food source is likely facilitated by gut bacteria too.

Like us, insects also have gut bacteria that help them to digest food.

Currently, we are investigating species diversity and specificity in a little studied subterranean ant-homopteran-microbe mutualism in the North Eastern US. Three species of 'fuzzy' ants (*Lasius umbratus*, *L. nearcticus* and *L. claviger*) tend multiple species of *Prociphilus* aphids and mealybugs in their underground nests. Both ants and homopterans are obligately involved in this interaction: they cannot live without each other. Species specificity of these interactions remained unknown thus far: do particular ants only tend particular aphids or mealybugs? And do these ants harbor specialized bacteria that allow them to feed only on the honeydew produced by aphids harboring specific *Buchnera*?

Barcoding now allows us to determine biodiversity in a hitherto little studied subterranean system.

Both questions can now be answered with the help of DNA barcoding techniques. First, by combining barcode data with morphological species identification of the same insect specimens, we can genetically verify earlier species identification and identify 'cryptic' species (species that were previously not recognized as different species, but can be told apart based on their barcodes). Second, the use of 'metagenomic barcoding' allows us determine the complete bacterial diversity inside an insect's gut in a single sequencing run.

The first species identification results, based on the combined genetic and morphological data, indicate that there likely is at least one cryptic *Prociphilus* species in the system, bringing the total number of aphid species to at least four. These first results show varying species specificity in the system: whereas *L. umbratus* is found tending all species, *L. claviger* predominantly associates with a single aphid species.

Preliminary microbiome results confirm that all *Prociphilus* aphids harbor *Buchnera* symbiotic bacteria and that specific *Buchnera* strains are associated with particular *Prociphilus* species. Also the mealybugs harbor bacteria, with mealybugs living in the same ant nest sharing bacterial strains.

Lastly, our analyses show that the ants themselves have relatively few gut bacteria (compared to other ants). Two ant guts contained *Gluconobacter*, which is known from sugary environments. Whether *Gluconobacter* is indeed common in fuzzy ants and plays a role in the digestion of honeydew remains to be further investigated.

In conclusion, barcoding allows us to unravel species diversity and specificity in a mutualistic interaction network of ants, aphids, mealybugs and their gut microbiomes. Once the system has been completely characterized, it might become a powerful new model system for the roles of microbes in insect nutritional mutualisms.

***Lasius claviger* specimen, point mounted for morphological species identification.**

Image credit: Christoph von Beeren

Fluorescent microscope image of an ant gut.

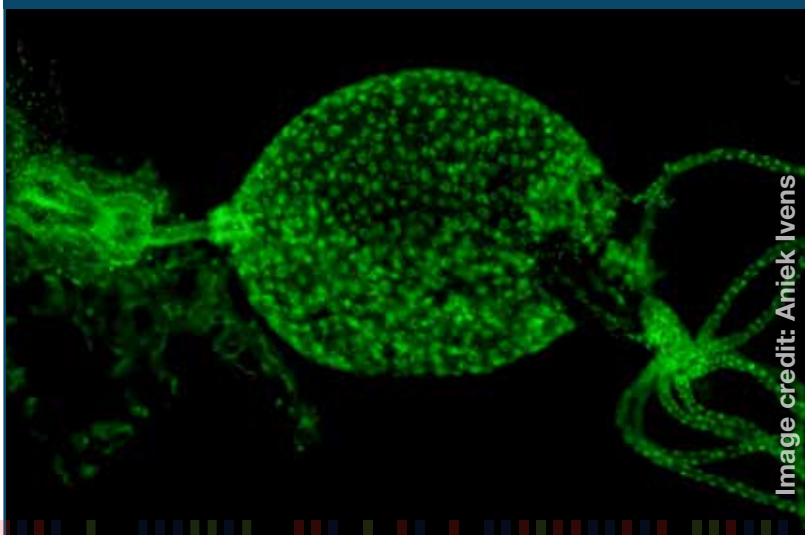


Image credit: Aniek Ivens

Elysia clarki on the green algae *Penicillus capitatus*. Image credit: Nick Curtis and Ray Martinez

Reconstructing the Diet of a Photosynthetic Sea Slug

Written by: Michael Middlebrooks (University of Tampa), Susan Bell (University of South Florida), Nicholas Curtis (Ave Maria University), and Sidney Pierce (University of South Florida)

A number of species of sacoglossan sea slugs are able to photosynthesize. They do so via kleptoplasty, a process where they feed upon coenocytic green algae and then sequester and maintain the algal chloroplasts within their own cells. Several species are able to use those sequestered chloroplasts to photosynthesize for many months. Our studies focus on *Elysia clarki*, a sacoglossan which can photosynthesize for 3-4 months after feeding. The slug is endemic to the Florida Keys and feeds on a variety of closely related rhizophytic green algal species.

Because *E. clarki* is able sequester chloroplasts from its algal food, we are able to use DNA barcoding of the *rbcL* chloroplast gene to reconstruct the slug's diet. This technique is quite valuable as the diets of specialist herbivores have often times been inferred, sometimes incorrectly, by an animal's proximity to potential food sources.

Barcoding uncovers mismatch between the diet and spatial association of a photosynthetic sea slug.

Therefore, we were interested in examining the spatial association of *E. clarki* with potential food sources, and then comparing those associations to their diets, which we reconstructed via DNA barcoding.

We performed intensive field surveys at a variety of sites across the slug's geographic range in the Florida Keys. *E. clarki* can be found in near-shore, low-wave-energy habitats such as mangrove swamps, but it is also found in human altered habitats such as borrow pits and canals. Our surveys covered habitats at two separate mangrove swamps, a borrow pit, and a mooring canal.

We found that in many instances the slugs were generally not found within close proximity to algal food sources and were most frequently found on bare substrate or in association with non-food algae.

Barcoding revealed that the slugs were often feeding on species of algae that were not detected during field surveys. This suggests that their spatial associations were poor predictors of diet. Only at one of the field sites were slugs commonly associated with an algal food source. At that site, DNA sequences only revealed a single algal species in diets of those slugs. Other sites, however, showed a much higher diversity for the diet of *E. clarki*, and many slugs had chloroplasts from multiple species of algae. A total of 17 distinct algal food sources were found from the survey. The barcoding revealed 13 new distinct algal food sources that have been previously unreported in the diet of *E. clarki*, although many of these sequences could not yet be resolved to the taxonomic level of species.

By combining the information garnered from dietary barcoding with that of field surveys, we were able to demonstrate that *E. clarki*'s spatial associations with algae are not accurate predictors of the slug's diet or its dietary selectivity. This is quite likely due to the slug's ability to photosynthesize and thus avoid or delay starvation during food shortages or while traveling between areas with inadequate food. Ideally, similar methods will be employed to examine the diets and ecology of other photosynthetic sacoglossans.

For more information about the results discussed in this article, see DOI: 10.1007/s00227-014-2431-9

Image credit: Michael Middlebrooks and Sidney Pierce

***Elysia clarki* on the green algae *Bryopsis plumosa*. The coloration of the slug can vary depending on the algal food source.**

Mangrove swamp field site in the Florida Keys where *E. clarki* is found.

Image credit: Sidney Pierce

Image credit: flickr.com/photos/8989278@N03/

DNA Barcoding Poisonous Plants

Written by: Massimo Labra (University of Milano-Bicocca)
Photos by: Andrea Moro

Exposures to toxic plants are the most frequent cause of poisonings that are reported to poison control centers. Although most of the cases of plant exposures occur in children, due to the accidental ingestion of showy fruits, the new trend of nutritional therapies and phytotherapy increases the poisoning cases in adults too.

Most people believe that plants are naturally safe and can be used as healing remedies for human health. This belief often leads to the spontaneous use of plants for medicinal and aesthetic purposes without evaluating the effects of their secondary metabolites on humans.

Moreover, confusion of an edible plant with a toxic one is another source of poisoning as suggested by the medical staff of "Ca' Granda" Hospital of Milan (Italy). Among the most relevant cases is the exchange of Alpine Sow-thistle (*Lactuca alpine* (L.) Wallr.) with the toxic *Aconitum* spp..

A correct plant identification is of essential importance for clinical diagnosis. Currently, toxic plants are identified on the basis of a patient's symptoms and morphological analysis of the ingested fragments. However, in most cases the plant samples are partially digested and therefore morphologically unrecognizable.

"A correct plant identification is of essential importance for clinical diagnosis."

In 2010, ZooPlantLab® at the University of Milano-Bicocca (www.zooplantlab.btbs.unimib.it) proposed the use of DNA barcoding techniques to identify poisonous plants. Results suggested that core barcode markers (*rbcL* and *matK*) combined with an additional spacer region *trnH-psbA* were able to distinguish poisonous plants from edible ones.

A dedicated BOLD project (ZPLPP) was created by the research group of ZooPlantLab®. The team developed a dedicated reference database including morphological and molecular data of poisonous species.

To support the diagnostic analysis at the poison centers, FEM2-Ambiente S.r.l. (www.fem2ambiente.com), a start-up born from the ZooPlantLab®, developed a molecular protocol for fast analysis of small plant fragments based on DNA barcoding.

Sequence Characterized Amplified Regions (SCARs) were identified starting from the DNA barcode sequences of poisonous plants. These markers were used to identify poisonous species by Real Time Polymerase Chain Reaction (rt-PCR). The tests were set up on the two most dangerous and frequently reported species: *Atropa belladonna* L. and *Colchicum autumnale* L.

A. belladonna L. (Fig. A) produces tropane alkaloids (atropine, hyoscyamine and hyoscine) with anticholinergic properties. Unfortunately, the fruits of this species are often misidentified as blueberries (Fig. B. - *Vaccinium myrtillus* L.) and other poisonous plants such as *Solanum nigrum* L. (Fig. C)

"...developed diagnostic kits for hospitals and poison centers..."

C. autumnale L. (Fig. D) contains an alkaloid called colchicine, which blocks cell division by inhibiting mitosis. This species is accidentally ingested as bear's garlic (Fig. E). Several species of the genera *Allium* and *Colchicum* have similar leaves and for this reason they could be mistakenly identified.

Results demonstrated that the use of specific SCARs combined with the analysis of PCR melting curves, enable the clear detection of poisonous plants. Thanks to this research, the team of FEM2-Ambiente S.r.l. have developed diagnostic kits for hospitals and poison centers to obtain a rapid response in cases of poisoning.

For more information about the results discussed in this article, see DOI: 10.1080/11263504.2014.941031

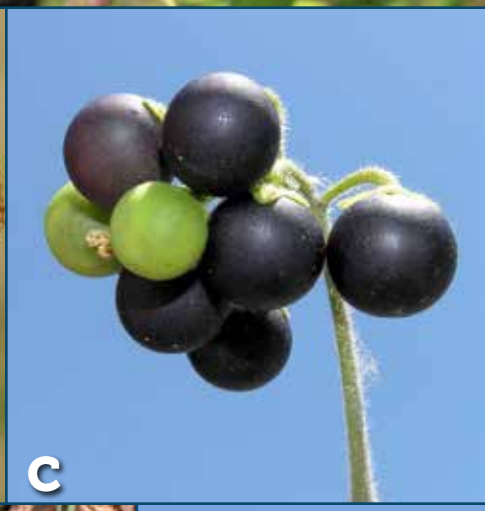


Image credit: Massimo Labra

***Atropa belladonna* L., also known as deadly nightshade, is an extremely toxic plant bearing a fruit that is easily mistaken for a blueberry. Eating as few as two to five berries can be fatal to a human adult.**

Research

Top 10 DNA Barcoding Publications 2014

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

1. Wells JD, Škaro V (2014) Application of DNA-based methods in forensic entomology in *Forensic DNA Applications: An Interdisciplinary Perspective*, ed. Dragan Primorac, Moses Schanfield, CRC Press, Boca Raton.

2. Bocak L, Barton C, Rampton-Platt AC, Chesters D, Ahrens D, Vogler AP (2014) Building the Coleoptera tree-of-life for >8000 species: composition of public DNA data and fit with Linnaean classification. *Systematic Entomology*. 39: 97-110.
DOI: 10.1111/syen.12037

3. Kelly RP, Port JA, Yamahara KM, Crowder LB (2014) Using environmental DNA to census marine fishes in a large mesocosm. *PLOS ONE* 9(1): e86175.
DOI: 10.1371/journal.pone.0086175

4. Hamilton CA, Hendrixson BE, Brewer MS, Bond JE (2014) An evaluation of sampling effects on multiple DNA barcoding methods leads to an integrative approach for delimiting species: A case study of the North American tarantula genus *Aphonopelma* (Araneae, Mygalomorphae, Theraphosidae). *Molecular Phylogenetics and Evolution* 71: 79–93.
DOI: 10.1016/j.ympev.2013.11.007

5. Piñol J, San Andrés V, Clare EL, Mir G, Symondson WOC (2014) A pragmatic approach to the analysis of diets of generalist predators: the use of next-generation sequencing with no blocking probes. *Molecular Ecology Resources* 14: 18–26.
DOI: 10.1111/1755-0998.12156

6. De Barba M, Miquel C, Boyer F, Mercier C, Rioux D, Coissac E, Taberlet P (2014) DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. *Molecular Ecology Resources* 14: 306–323.
DOI: 10.1111/1755-0998.12188

7. Techen N, Parveen I, Pan Z, Khan IA (2014) DNA barcoding of medicinal plant material for identification. *Current Opinion in Biotechnology* 25: 103–110.
DOI: 10.1016/j.copbio.2013.09.010

8. Joly S, Davies TJ, Archambault A, Bruneau A, Derry A, Kembel SW, Peres-Neto P, Vamosi J, Wheeler TA (2014) Ecology in the age of DNA barcoding: the resource, the promise and the challenges ahead. *Molecular Ecology Resources* 14: 221–232.
DOI: 10.1111/1755-0998.12173

9. Moravec F, Justine JL (2014) Philometrids (Nematoda: Philometridae) in carangid and serranid fishes off New Caledonia, including three new species. *Parasite* 21:21.
DOI: 10.1051/parasite/2014022

10. Li X, Yang Y, Henry RJ, Rossetto M, Wang Y, Chen S (2014) Plant DNA barcoding: from gene to genome. *Biological Reviews*.
DOI: 10.1111/brv.12104

Credits and Contributions

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