

international
BARCODE
OF LIFE



Barcode BULLETIN

June 2016
Vol.7, Issue 2

Announcing the 7th International Barcode of Life Conference

Research

Uncovering
Cryptic
Diversity
Worldwide

Applications

Host
Specificity,
Barcoding, and
Social Media

Image credit: Olivier Maurin

News Briefs

The first special issue from the 6th International Barcode of Life Conference will appear in *Philosophical Transactions B*, with full open access, on August 1, 2016.

From August 28 to September 1, 2016, the 32nd Symposium of the European Society of Nematologists will take place at the University of Minho, Braga, Portugal. The symposium is a venue for multidisciplinary collaborations, with session topics ranging from “Species delineation, molecular barcodes and diagnostics” to biocontrol, invasive species, and climate change. For more information about the scientific programme, important dates, and registration, visit the conference website at esn2016braga.com.

The International Conference on DNA technology for authentication, quality control, and conservation of herbal material will be held in Hong Kong from December 12 to 14, 2016. The abstract submission deadline is June 30, 2016, and the registration deadline is August 31, 2016. For more information, visit the conference website at www.bch.cuhk.edu.hk/ic2016/.

The **School Malaise Trap Program** team would like to thank the following people and organizations for their support through a recent crowdfunding campaign: Antarctic Biodiversity Portal; Bradley Zlotnick, MD; Coastal Marine Biolabs; Crystal Sobel; Dr. Mary Ann Hawke; Eldon Eveleigh; J. Pollock; J. Watty; John Hannah; Nancy Green; Nicole Anthony; Rebecca Lee; Rick & Gayle Prosser; Rob & Carla Guthrie; Sandra Berrios Torres, MD; Susan Robinson; Teresa Allan; the Ruiters; The York School Science Department; and ten anonymous donors.



Image credit: Olivier Maurin

Welcome to our June 2016 issue of the Barcode Bulletin.

As you might have guessed from the front page, we are already looking ahead to the next international conference, which will be hosted by South Africa at a spectacular venue.

But there is far more to read, e.g. part 2 of our ABS series, a number of insect stories on mosquitos, bee-flies, moths, and army ants, and much more.

Enjoy reading,

Dirk Steinke
Editor-in-chief

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7th International Barcode of Life Conference

Written by: Michelle van der Bank on behalf of the Local Organizing Committee and the International Scientific Collaboration Committee (ISCC)

The African Centre for DNA Barcoding (ACDB) and the University of Johannesburg (UJ) are proud to announce and welcome delegates to our hosting of the 7th International Barcode of Life (iBOL) Conference, from November 20 to 24, 2017. This is the first time that this event will be held on the African continent. The venue for the hosting of this prestigious event will be the Nombolo Mdhluli Conference Centre, Skukuza, located within the heart of African wildlife at Kruger National Park (KNP), South Africa.

The Centre is capable of hosting up to 700 delegates, with a number of smaller rooms for plenaries and workshops, and is in walking distance from the accommodations. The KNP was established in 1898 and is the flagship of the South African National Parks. It covers an area of nearly 2 million hectares and is home to the big five as well as a wide range of other species, which includes 336 trees, 49 fish, 34 amphibians, 114 reptiles, 507 birds, and 147 mammals. Skukuza Rest Camp is the largest camp in the KNP, well situated in the heart of



'Big 5' territory, 10 km from the Paul Kruger Gate. The rest camp is fenced-in, making it safe to move about freely, and overlooks the Sabie River, where big game such as elephants, hippopotamuses, and buffaloes are often found; there is also a high concentration of predators like lion, hyena, and leopard in the vicinity of the camp.

Accommodation at Skukuza Rest Camp caters to a variety of guest requirements. You can choose anything from luxury, river-view bungalows and self-catering bungalows to two-bedded tents (communal ablutions) and campsites. All accommodation units also have their own barbecue facilities. For those who prefer to stay outside the Park, the four-star Protea Hotel Kruger Gate, situated 100 m from Paul Kruger Gate and 10 km from Skukuza, provides great-value accommodation.

"Our intention is to make this a global conference with a distinctive African flavour..."

Delegates/spouses don't need to be concerned about how to fill their free time; all typical activities associated with Kruger Park are to be found in Skukuza or nearby. These include early morning and sunset drives as well as evening game drives to view Kruger's nocturnal animals on the back of an off-road vehicle in the company of experienced guides; bushwalks for the more adventurous where you will track rhino, elephant, and lion on foot; a round of golf at the Skukuza Golf Course situated on the outskirts of Skukuza Rest Camp; birdwatching at the Lake Panic Bird Hide located 7 km outside the camp; a visit to the Stevenson Hamilton Memorial Library; or just relax next to the pool and enjoy the wonderful South African sunshine. For those that want to stay a few days longer in the Park, we highly recommend the wilderness trails (Bushman, Mathikithi, Napi, Nyalaland, Olifants, Sweni, and Wolhuter). These trail excursions are two days in length with three overnights in rustic huts; booking well in advance is strongly advised. More information on post-conference tours will be made available on the conference website (www.dnabarcodes2017.org).

Our intention is to make this a global conference with a distinctive African flavour, using the event to highlight, support, and encourage African researchers across the continent, and to link them up with the global barcoding network. The conference format will include plenary lectures, parallel sessions comprising both invited talks and contributed talks, and poster presentations. The optional first day (November 20) will be devoted to training workshops. Several student travel bursaries (developing nations) will be made available; see the conference website for more details.

The 7th International Barcode of Life Conference will undoubtedly offer delegates and guests alike an unforgettable networking experience, with a backdrop not found anywhere else on the planet. The prospect of hosting world-leading delegates and experts in the field under African skies is a truly thrilling one, and we are looking forward to welcoming you all soon in South Africa.



Image credit: sanparks.org



Image credit: sanparks.org

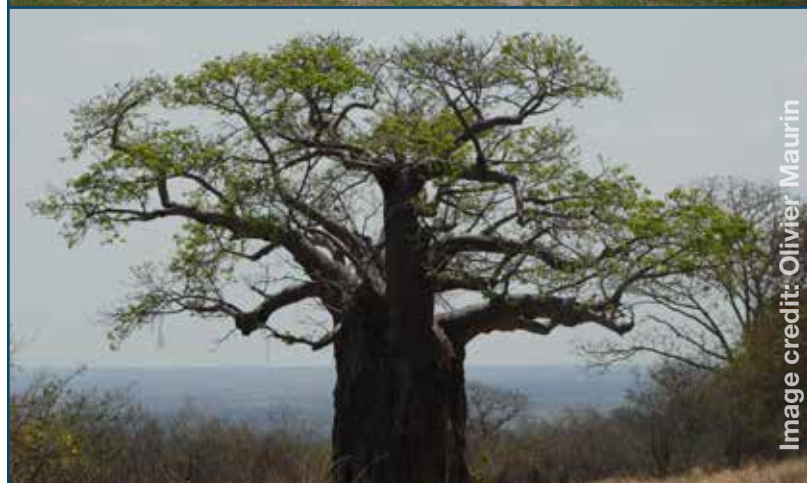


Image credit: Olivier Maurin

Important dates

Deadline for submission of abstracts:
July 1, 2017

Notification of acceptance of abstracts:
September 20, 2017

Deadline for early-bird registration:
September 30, 2017

Deadline for online registration:
November 15, 2017

Exploring the Guests of Army Ants

Written by: Christoph von Beeren (Technical University Darmstadt, Germany)
and Daniel Kronauer (The Rockefeller University, USA)

An unforgettable moment for many tropical biologists is their first encounter with an army ant swarm raid, in which a carpet of hundreds of thousands of individual ants roams the forest floor in search of live prey. Inspired by their impressive warfare, Henry Walter Bates wrote about army ants: "Wherever they move, the whole animal world is set in commotion, and every creature tries to get out of their way". Well, not entirely... A diverse assemblage of arthropod species does just the opposite: they seek close contact with army ants, and take advantage of the immense resources that army ant colonies provide. These guest species are the focus of our research.

During the last few years, we have started to systematically survey the guest community of the six local *Eciton* army ant species at La Selva Biological Station in Costa Rica. This community includes diverse arthropod groups such as beetles, mites, bristletails, flies, wasps, and millipedes. Among the guests are some peculiar creatures, many of which are highly adapted to life with ants. For example, a drop-shaped, or limuloid body form, along with short, retractable appendages, can be found in many army ant guests, ranging from different beetle taxa, such as the genus *Vatesus* (see image on next page) to silverfish. This general gestalt

probably makes it difficult for the ants to grasp the guests with their mandibles. The most bizarre guests are likely those that closely resemble the appearance of their host ants, and also mimic their behavior (see image above, which shows an *Ecitophya* beetle in a raid column of the army ant *Eciton burchellii*). This form of mimicry has evolved independently in numerous rove beetles, probably in response to selection pressure from visual predators like army ant-associated birds, which attend the swarm raids and feed on the arthropods that try to escape from the ants.

DNA barcoding is key for the success of this project because a large proportion of guest diversity is still unknown, and

the taxonomy of many groups is unsettled so that morphological identification is not possible in a timely manner. DNA barcoding therefore provides a quick and objective approach to determine possible species boundaries. Distinct barcode clades are then carefully evaluated by morphological inspection in collaboration with taxonomic experts, and are sometimes further studied with additional nuclear genetic loci. Applying this integrative approach has resulted in the discovery of 61 *Eciton*-associated guest species, about 12 of which represent new, often cryptic species.

"The correct identification of species is vital for determining host specificity, which is a key parameter when analyzing host-symbiont community structure."

The correct identification of species is vital for determining host specificity, which is a key parameter when analyzing host-symbiont community structure. DNA barcoding is

also pivotal for matching adult and immature stages. By matching larval and adult life stages in *Vatesus* rove beetles, for example, we were able to, for the first time, reconstruct the life cycle of an army ant guest. It turns out that by synchronizing their reproduction and larval development with their host ants, *Vatesus* beetles track the stereotypical colony cycles of *Eciton* army ants, which alternate between stationary and nomadic phases. Since our knowledge of the basic biology of army ant guests is very restricted, it seems likely that many more such fascinating adaptations await scientific discovery.

“...it seems likely that many more such fascinating adaptations await scientific discovery.”

By assessing army ant guest diversity and by providing tools for easy and reliable species identification via DNA barcodes, we are setting the baseline for studies targeting the ecological

and evolutionary dynamics in these species-rich host-symbiont communities. Hopefully, additional researchers will eventually join us in exploring the fascinating microcosm of army ant guests.

For more information about the results discussed in this article, see DOI: [10.1111/mec.13500](https://doi.org/10.1111/mec.13500)



Image credit: Daniel Kronauer

DNA barcodes allowed us to match adult *Vatesus* beetles (left) with their larval stages (right). Both participate in colony emigrations.



Image credit: Daniel Kronauer

Unexpected Cryptic Diversity in Socotra's Reptiles

Written by: Raquel Vasconcelos (Universidade do Porto, Portugal, and Universitat Pompeu Fabra, Spain); Xavier Santos (Universidade do Porto, Portugal); Santiago Montero-Mendieta, Marc Simó-Riudalbas, and Salvador Carranza (Universitat Pompeu Fabra, Spain); Roberto Sindaco (Museo Civico di Storia Naturale, Italy); Mauro Fasola and Edoardo Razzetti (Università degli studi di Pavia, Italy); and Gustavo Llorente (Universitat de Barcelona, Spain)

A study led by researchers from the Spanish National Research Council (CSIC) finds that over 50% of the reptile species in the archipelago of Socotra could still be undiscovered.

Astonishing biodiversity

Located in the north-west Indian Ocean and comprising four small islands, Socotra (a governorate of Yemen) is considered one of the most difficult to access and distinct archipelagos in the world. Regarded as the “jewel” of biodiversity in the Arabian Sea, the Socotra Archipelago was designated a UNESCO World Heritage Natural site in 2008 because of its high level of endemic species. The long isolation period from continental Arabia (≈ 20 million years ago), together with its topography and its high ecological and climatic diversity, have given rise to exclusive and spectacular endemic fauna and flora found nowhere else on Earth. Biological field surveys have counted nearly 700 endemic species, including 35% of its 825 plant species and 95% of its more than 100 land snail species.

Despite the richness and uniqueness of Socotra's biodiversity, increasing and insufficiently controlled human activities (e.g. cattle and sheep farming, introduction of exotic species,

unsustainable exploitation of resources, and infrastructure and tourism development) pose a serious threat to the long-term conservation of its unique biodiversity.

Tool for conservation

With few endemic mammal or bird species and no endemic amphibians, reptiles constitute the most relevant vertebrate fauna of Socotra with 31 presently recognized species, 29 of which are endemic (94%). Due to their abundance in Socotra, reptiles constitute a keystone group in the trophic system, both as insect predators and as prey for birds. Moreover, some species have strict associations with specific habitats, as in the case of the endemic gecko *Hemidactylus dracaenacolus*, which is found only on the endemic Dragon's blood tree (*Dracaena cinnabari*); both species are critically endangered.

In this regard, the construction of a DNA-based reference library for all reptiles of the Socotra Archipelago can serve as an integrative tool for monitoring its biodiversity.

“...increasing and insufficiently controlled human activities... pose a serious threat to the long-term conservation of its unique biodiversity.”

Since 2007, a team led by Salvador Carranza (senior researcher at the Institute of Evolutionary Biology in Barcelona) has conducted several expeditions to Socotra in order to collect reptile samples (1 mm of tail tips) from all of the currently known localities. They have successfully sequenced a 663bp region of cytochrome c oxidase 1 (COI) for 380 individuals, representing all currently recognized reptile species in the Socotra Archipelago.

The results of this research will be very useful for monitoring reptile biodiversity in Socotra and establishing conservation priorities. "Our main goal was to generate DNA barcodes for all Socotran reptiles, and so provide a valuable tool for quick specimen identification by airport / port authorities. However, we also found unexpectedly high levels of cryptic diversity within the reptiles of the Socotra Archipelago," said Salvador Carranza.

Cryptic diversity

The study, published in PLoS ONE in March, claims that up to 54% of the reptile species in Socotra might still be undescribed. "Considering that currently a third of Socotran reptiles are threatened, the results of our work could have major implications for species conservation," adds Raquel Vasconcelos, postdoc researcher at the CIBIO-inBIO, Portugal.

Although short-length markers such as COI are often time- and cost-effective proxies for specimen identification and species discovery (especially of cryptic species), they frequently are not representative of the full evolutionary history of species. "We propose that the taxonomy of Socotran reptiles should be revised using an integrative framework incorporating multiple loci, morphology, and ecological data," commented Santiago Montero-Mendieta, currently a PhD student at the Estación Biológica de Doñana, Seville.

For more information about the results discussed in this article, see DOI: [10.1371/journal.pone.0149985](https://doi.org/10.1371/journal.pone.0149985)



The endemic gecko *Hemidactylus dracaenacolus*, above, is only found on the endemic Dragon's blood tree (*Dracaena cinnabari*), below.



The bee-fly emerged from its pupal exuvia and its host pupa, leaving two empty skins.



Barcode
Applications

Image credit: Lars GR Nilsson

A Bee-fly's Host, Facebook, and DNA Barcoding

Written by: Johannes Bergsten (Swedish Museum of Natural History, Sweden)

The public's interest in insect identification has increased massively in recent years for several reasons. First, macro-photography has become available and possible for everyone to use – even a mobile phone can be enough. Second, the internet has opened up insect identification as a hobby for anyone. In the past, you had to be a collector of hard-to-get identification literature with a whole library at home, but no longer. While sometimes unreliable, many insect species can be identified from photos. Finally, social media like Facebook provide a forum where like-minded people can find each other, share identification tips, post images, and answer each other's questions. In Sweden alone, there are Facebook groups for every major insect order, with hundreds of members in each. Professionals, amateurs, and the general public are collectively engaged in discussing identifications and biology as photos are shared. Serendipitous discoveries lie around the corner.

Last summer, a six-year-old boy named Casper found a pupa in a garden in southernmost Sweden, and, via his mother and a friend, it was photographed and posted in a Facebook group. The Facebook community replied that it was a pupa of an owl moth (Noctuidae), but no one

could identify it further. The pupa was therefore kept in a jar to let it hatch. Five weeks later, the jar was opened and the pupa had hatched but, instead of a moth, a large golden-haired bee-fly had emerged.

A photo of the fly was taken before it was released (see above) and posted under the same Facebook thread asking for

ID help. The bee-fly was tentatively identified as *Villa hottentotta* or *V. moesta*, but replies also pointed out that this was an interesting observation and that host records were scarce for bee-flies. However, the owl moth was still unidentified and had been killed by the parasitoid; all that remained were two pupa exuviae.

This is where DNA barcoding came in. One of the many advantages of DNA barcoding is that it works equally well at any life stage, including those where morphological keys are lacking. It also works with different kinds of remains as long as there is DNA left and it is not too degraded.

“Professionals, amateurs, and the general public are collectively engaged in discussing identifications and biology...”

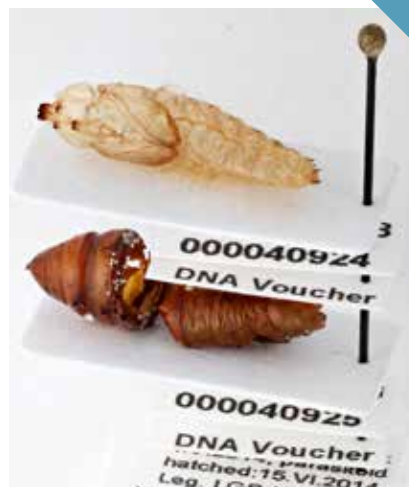
Both pupal exuviae were sent to the Swedish Museum of Natural History, where DNA was extracted at the Centre for Genetic Identification (CGI). The standard COI barcode fragment was amplified, and the Barcode of Life Data Systems (BOLD) was used to compare both barcodes to the reference library. For the bee-fly pupa,

The heart and dart moth “has never previously been recorded as a host for this species of bee-fly.” reference sequences produced by NORBOL (Norway) and FINBOL (Finland) were essential, and, thanks to these, the pupa could be identified as *Villa hottentotta*. So who was the host? For butterflies and moths, thanks especially to the efforts of FINBOL and GBOL (Germany), over 90% of the species occurring in Sweden are covered in BOLD. The owl pupa was identified as the heart and dart moth, *Agrotis exclamatoris*. This is a common moth in Sweden but, as the literature on bee-fly hosts indicates, it has never previously been recorded as a host for this species of bee-fly.

New knowledge has been produced thanks to the curiosity of a six-year-old who found a pupa, the active interest of the Facebook community, and DNA barcoding, which provided the tool for identifying pupal remains. It turns out that bee-flies are not very specific in their host selection. Hosts are most often wasps, bees, beetles, moths, butterflies, flies, or lacewings, but the egg cases of grasshoppers, cockroaches, and spiders also serve as food for some species. For most bee-flies, the choice of host is based, not on the particular species but, on what is available in the substrate zone.

Based on the following article:

Bergsten J, LGR Nilsson and R Bukontaite. 2015. The noctuid moth *Agrotis exclamatoris* identified as a host for the bee fly *Villa hottentotta* using DNA barcoding (Diptera: Bombyliidae) [In Swedish]. *Entomologisk Tidskrift* **136**: 121-130.



Above: Both pupal remains were barcoded and vouchers kept.

Below: The heart and dart moth that never developed.



Below: The pupal skin of the bee-fly *Villa hottentotta* holding its DNA barcode.



ABS for DNA Barcoders, Part 2: Recipes for Compliance

Written by: Dirk Neumann (Zoologische Staatssammlung München, Germany)
and Kate Davis (Independent ABS Consultant, Canada)

Following from Part 1 in the March 2016 issue, where we introduced the ingredients of access and benefit-sharing (ABS) for nourishing research partnerships.

The many Parties to the Convention on Biological Diversity (CBD) and the Nagoya Protocol (NP) have developed a challenging variety of legal approaches that researchers and institutions must respect. When planning projects or applying for funding, researchers should allow sufficient time for negotiations with providers to develop the necessary partnerships, detail the collecting / utilising / transferring of samples and publishing of results, and consider the additional expenses needed.

It is worth noting that many countries have established access measures under the CBD but will not ratify the NP in the near future, and that some genetic resources (GRs) – even if originating in a NP Party – might fall outside the scope of compliance laws in the countries where they are utilised, so tracking provenance and utilisation are vital to demonstrate researchers' own due diligence.

The NP's [ABS Clearing House](#) will hold details of National Focal Points (information

on national ABS measures) and Competent National Authorities (responsible contacts that grant, or coordinate, access). Unless a providing country grants free access, Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) come into play. *Prior informed* consent involves explaining the research and how material will be used to the appropriate authority(/ies) before access takes place. PIC might be granted by a national authority, a state/provincial authority, private landowners, local communities, or a combination; it might be set out in a permit or a longer agreement. MAT may be combined with PIC and may involve the same or different actors (e.g. institutional partners rather than authorities); it is essentially a contract between provider and user setting out how material may be used and which kinds of benefits (see [Annex of NP: Monetary and Non-Monetary Benefits](#)) will be shared. Establishing MAT often involves developing a collaborative research agreement with national partners. Because MAT may include commitments that exceed the lifespan of funded projects or research positions, institutions are advised to appoint staff that are authorised to conduct negotiations, sign agreements, and ensure their implementation.

NP Parties must develop compliance measures for its users of GRs and monitor GR utilisation. Countries are required to establish one or more checkpoints to monitor legal access and utilisation of material acquired after the coming into force of the NP, e.g. by checking original PIC and MAT. Additionally, the EU regulation on ABS

The Nagoya Protocol emphasises compliance: researchers need to keep records of PIC, MAT, utilisation, transfers, and benefits.

obliges users to demonstrate due diligence and to 'keep the information relevant to access and benefit-sharing for 20 years after the end of the period of utilisation' of GRs utilised inside the EU. However, if the samples are kept or not completely consumed, there is no end point. Thus users are well advised to keep utilised GRs documented and traceable in bio-repositories beyond the life span of individual research projects or careers.

User compliance laws may not distinguish between users from industrialised or developing countries. While some biodiverse developing countries and researchers from those countries identify themselves as 'providers' rather than 'users', e.g. when sending samples for identification / barcoding abroad, the laws in user-heavy countries (e.g. EU Member States) may require evidence that PIC and MAT have been established. Researchers and collections institutions are strongly advised to keep permits and record any ABS-related terms (or their absence) associated with GRs and sequence data, to document the provenance of the samples, and to keep track of benefits shared. If PIC and MAT allow for transfer to other parties, the terms (including any unique identifiers) also must be transferred, using a Material Transfer Agreement (MTA). PIC and MAT should also cover uploading of sequence data to public domain databases.

The NP provides room for voluntary measures. The collections networks of the Consortium of European Taxonomic Facilities (CETAF), the Global Genome Biodiversity Network (GGBN), and Botanic Gardens Conservation International have developed new recipe books: voluntary codes, models, and guidance to help institutions and researchers to understand ABS and revise internal policies and procedures. The CETAF and GGBN packages are harmonised, and CETAF currently seeks recognition of their Best Practice under the EU law. Barcoding institutions should use these tools to harmonise ABS policies and thus try to establish a global standard for non-commercial GR utilisation for biodiversity research and monitoring. Doing so would help to build the trust necessary for countries to establish practical simplified access and monitoring measures to achieve the CBD's goals.

A new cookbook: The CETAF package includes the code of conduct, best practices, and model documents.

ACCESS
&
BENEFIT-
SHARING

ABS

CODE OF CONDUCT & BEST PRACTICES



Exploring and Documenting Diversity in Nature



includes "MATERIAL TRANSFER AGREEMENTS" TEMPLATES

To See a World in a Gram of Soil

Written by: Nicole Fahner and Mehrdad Hajibabaei (University of Guelph, Canada)

The Peace-Athabasca Delta is the world's largest inland freshwater delta, a designated "wetland of international importance" under the UN's Ramsar Convention, a UNESCO World Heritage Site, and part of Wood Buffalo National Park, which is Canada's largest national park. Like all wetlands, the delta provides important habitat as well as many essential ecosystem services, including carbon sequestration, maintenance of food webs, and water quality improvement. Recently, however, the vast operations of nearby Alberta oil sands have raised concerns regarding the future integrity of this valuable wetland.

Monitoring biodiversity helps to inform us of the status or "health" of wetlands, and multiple wetland quality indices used by monitoring agencies are based specifically on plant diversity.

Aboveground plant surveys "only provide a snapshot of the site's total diversity and miss short-lived or dormant taxa."

Plant diversity at a site would conventionally be identified through an aboveground survey, but these surveys only provide a snapshot of the site's total diversity and miss short-lived or dormant taxa. Environmental DNA (eDNA) from soil, however, can originate from any plant tissues including seeds, pollen, detritus, and both active and dormant roots. This means that metabarcoding – the simultaneous identification of all members of a broad taxonomic group using DNA sequences – of soil eDNA may provide a more integrated view of local plant diversity from a single assessment than an aboveground survey. Our work as part of Biomonitoring 2.0, a large-scale project conducted in Wood Buffalo National Park, focused on developing this new approach for surveying vegetation.

As a new methodology, many different aspects of eDNA and metabarcoding need to be addressed to validate the results of these surveys and ensure that findings are robust, repeatable, and informative. There are technical considerations at all steps from sampling design and sample processing to molecular protocols and bioinformatic analyses that make this work challenging, yet the immense potential of this technology to provide new insights is exciting!

Our first study evaluated four DNA markers to determine which are best able to recover, resolve, and annotate vascular plant diversity from soil samples. Our findings (currently in review for publication) supported the use of the standard DNA barcode *rbcL*, along with ITS2, for plant metabarcoding.

Persistence of DNA in the environment after an organism dies is a common concern for those working with eDNA because long-term stability of so-called “zombie” DNA could mask short-term changes at sites. For our second study, we are looking at annual variability in our soil eDNA-based belowground plant assessments and comparing this with aboveground data in order to better understand the differences between belowground eDNA and aboveground vegetation dynamics.

Through the metabarcoding of eDNA, we can describe whole communities from just a sample of soil, water, or air, opening up all new possibilities to better understand and monitor ecosystems. DNA metabarcoding and eDNA are transforming many branches of ecological research, and, with continued study, this approach may ultimately become routine in biomonitoring programs.



Image credit: Donald Baird (retrieved from biomonitoring2.org/)

Above: These remote boreal wetland sites were the focus of the Biomonitoring 2.0 project.

Below: This example of metabarcoding output shows vascular plant diversity observed in the Peace-Athabasca Delta. *Chamerion angustifolium* (A), *Salix exigua* (B), *Moehringia lateriflora* (C), *Persicaria amphibia* (D), *Betula papyrifera* (E), *Orthilia secunda* (F), and *Sonchus arvensis* (G) are among the species identified from soil eDNA.

Image credits: John Haslam (A), Matt Lavin (B), Jason Hollinger (C), Joshua Mayer (D and F), Nicholas A. Tenelli (E), and Frank Mayfield (G). Images retrieved from Flickr under Creative Commons licenses.



CBG Training Program Launches New Website

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

Celebrating two years since its launch, the Research Training Program (RTP) in DNA Barcoding is pleased to announce its [new website](#). Delivered by the Centre for Biodiversity Genomics (CBG) at the Biodiversity Institute of Ontario, University of Guelph, the RTP offers a unique immersion experience in molecular biodiversity from the place of inception of DNA barcoding.

Remaining at the forefront of methodological development in DNA barcoding, the CBG is fulfilling its mandate to aid in building biodiversity genomics analytical capacity across the planet. The CBG has been hosting visitors from around the world since 2005, sharing its experience and latest methodological advancements; however, it was only in 2014 when a dedicated research training program was formally introduced. By partnering with the [online course in DNA barcoding](#) offered by the University of Guelph, the RTP complements theoretical background with solid practical experience.

The four-week program involves at least 120 hours of personalized hands-on instruction, in-depth discussions, and practical sessions supervised by a team of PhD-level researchers. Training timelines are adjusted so that at

least 2–6 people are enrolled simultaneously, working as a team, but allowing for personalized instruction. Activities take place in a designated visitor training lab, with guided “behind-the-scenes” tours to core operational units, including the Canadian Centre for DNA Barcoding – CBG’s state-of-the-art molecular analytical hub. This allows trainees to experience standard barcoding workflows in a medium-throughput lab setting and to observe their deployment and automation in the world’s largest high-throughput DNA barcoding analytical facility.

Training modules cover all aspects of the DNA barcoding pipeline, from best practices in collection management, through medium-throughput molecular analyses, to specialized informatics approaches – the full set of skills and knowledge that are a must-have for an advanced barcoder. This comprehensive skill set is further reinforced through discussions with CBG’s faculty, researchers, and core technical staff involved in the development and implementation of standardized operational workflows and protocols. Program participants receive a temporary visiting researcher appointment from the University of Guelph. After successful completion of the course, they receive a certificate from the CBG.

The RTP is well suited for research professionals, graduate students, and lab technicians with scientific background in biology who are considering DNA barcode applications. The instructors are committed to helping trainees meet their individual research targets, whether they are exploring the barcoding protocols for a specific group of organisms or gaining general knowledge to discover new barcoding applications. The small number of simultaneously trained participants makes the course highly customizable. The program venue also allows participants to bring and analyze their own materials during their training.

The launch of the RTP has been made possible due to contributions from partners and funders that facilitated the development of its individual modules and/or enabled the participation of international visitors through grants and contracts. Among them, the Conference Board of Canada supported a DNA barcoding capacity-building project in Peru in 2013-2015 under the CATRTA program, which facilitated the training of six Peruvian experts in Canada. In addition,

“...the course is highly customizable”, designed for trainees that are “exploring the barcoding protocols for a specific group of organisms or gaining general knowledge to discover new barcoding applications.”

the Secretariat for the Convention on Biological Diversity has thus far enabled the participation of 11 experts from developing countries, with funding support from the Japan Biodiversity Fund. These past and on-going partnerships have been instrumental in designing the Program and fine-tuning

it to help address key areas of application, such as detection and monitoring of invasive alien species, agricultural pest monitoring, and biodiversity surveillance.

The RTP is in the process of recruiting participants for 2016-2017 and welcomes inquiries from people and organizations interested in undertaking the course. Understanding that many prospective trainees may not have funding to facilitate their enrolment, the RTP welcomes contributions from donors and partnerships with organizations interested in sponsoring bursaries and scholarships for experts from lower-income countries, students, and other participants with limited funds.

For more information on the RTP, please visit <http://dnabarcoding.training>.



Felimida elegantula collected from Kerkennah Islands, Tunisia.

So Different Outside but so Identical Inside: the Case of a Sea Slug

Written by: Giulia Furfaro (Università "Roma Tre", Italy), Marco Oliverio (Università di Roma "La Sapienza", Italy), and Paolo Mariottini (Università "Roma Tre", Italy)

Felimida elegantula (Philippi, 1844) is a rare nudibranch that is considered endemic to the Mediterranean Sea and is likely one of the lesser-known sea slugs in the basin. It was recently redescribed based on specimens from Sardinia (Italy), with an assessment of its taxonomy and a review of the known morphotypes. When a single specimen of a very peculiar sea slug was collected from Djerba Is. (Gulf of Gabès, Tunisia), outside of the known range of *F. elegantula*, it was initially considered a hybrid of the species *Felimida purpurea* and *Felimida luteorosea*.

However, when we found a second specimen of this elusive nudibranch during a collecting expedition to the neighbouring Kerkennah Islands (Tunisia) in the summer of 2015, we identified it in the field as a color variant

of *F. elegantula*, with a strikingly divergent chromatic pattern.

The Tunisian specimen was an adult (15 mm) with a notum color array consisting of large irregular ovoid spots, delimited by a red edge with a

whitish opaque area enclosing a brilliant white central part. Some spots were separated by pink regions, while other spots were fused together, creating a complicated chromatic pattern. The Tunisian specimens fell well outside the known chromatic variability of *F. elegantula*. Anatomically,

while the specimen from Kerkennah revealed some differences, especially in the reproductive structures, the masticatory apparatus showed no significant variation from the other Mediterranean specimens studied thus far.

"This study... confirms that this elusive sea slug is an extremely variable species in both chromatic pattern and anatomy."

Given the uselessness of the internal anatomy for identification – a frequent occurrence with nudibranchs that could be due to either limited sampling or extreme variation – we also performed DNA barcoding to definitively assess the systematic position of the Tunisian specimen and to better understand the meaning of the observed morphological variation. Sequences were analyzed using Automatic Barcode Gap Discovery (ABGD), a distance-based method designed to detect the “barcode gap” in the distribution of pairwise distances, and species hypotheses were tested phylogenetically by Maximum likelihood analysis and Bayesian inference. Using a dataset of closely related species of the genus *Felimida*, we found that the ranges of intraspecific genetic distances within the putative species were always considerably lower (with a clear gap) than the smallest interspecific distances. The figure below shows the resulting tree, portraying the phylogenetic relationships among the assayed specimens based on the COI dataset.

This study supports our hypothesis that the Tunisian specimens were a new morphotype of *F. elegantula* and confirms that this elusive sea slug is an extremely variable species in both chromatic

pattern and anatomy. Furthermore, the geographical range of the species has been extended to the African coast of Tunisia.

For more information about the results discussed in this article, see DOI: [10.1007/s12526-016-0480-7](https://doi.org/10.1007/s12526-016-0480-7)



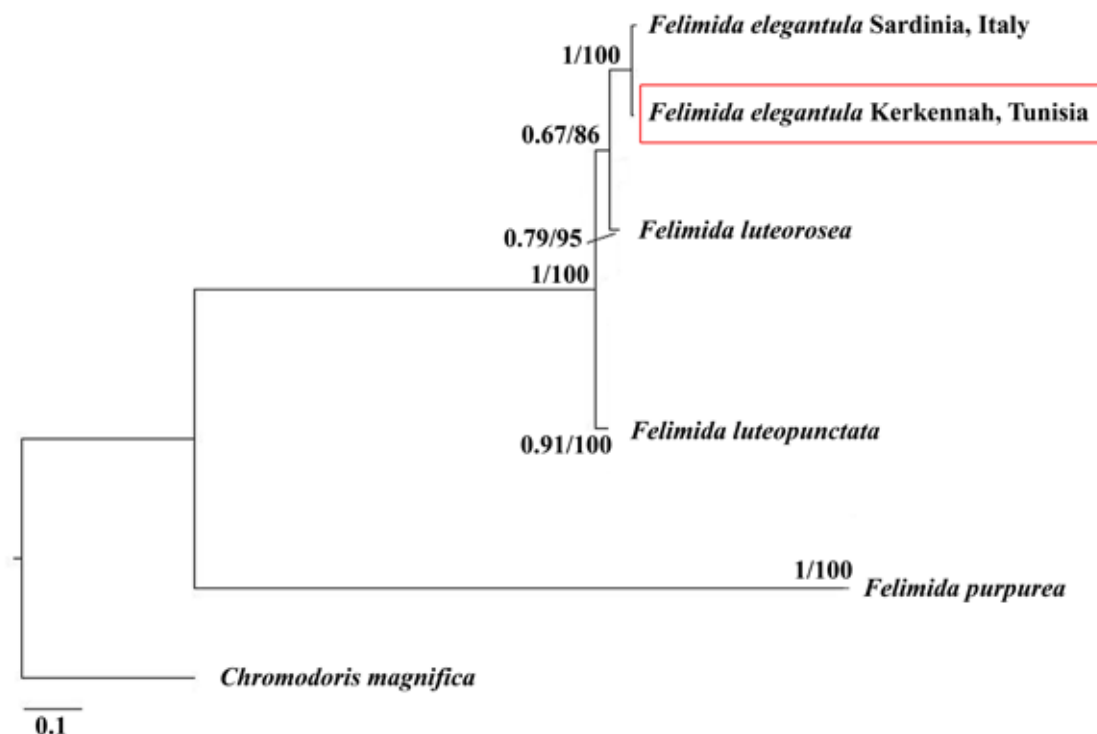
Above: The new distribution map of *F. elegantula*.

Below: *F. elegantula* specimen from Sardinia, Italy.



Image credit: Egidio Trainito

Phylogenetic tree with numbers at the nodes for Bayesian posterior probability and ML bootstrap support, respectively; the clearly monophyletic *F. elegantula* clade included the Tunisian sample.



DNA Barcoding Mosquitoes in Southeastern Australia

Written by: Jana Batovska (Department of Economic Development, Victoria, Australia)

Mosquitoes are a major health concern due to their ability to transmit pathogens that cause diseases such as malaria, dengue, and yellow fever. Mosquito surveillance programs help prevent these diseases by monitoring mosquito numbers and providing information about when and where viruses might be circulating. These programs trap mosquitoes near towns and cities, identify them, and then test them to see if any viruses are present. In order to identify mosquitoes, trained specialists look for morphological traits that distinguish species. However, certain species look very similar, and specimens can get damaged by the trapping process. A molecular method like DNA barcoding can complement mosquito identification by providing less-subjective species determinations.

In order to use DNA barcoding to enhance mosquito identification in the Victorian Arbovirus Disease Control Program (VADCP), a project was initiated to establish a barcode database for mosquitoes commonly found in southeastern Australia. The project used 113

mosquito specimens, representing 29 species and 12 genera, and two barcoding regions, COI and ITS2. A specimen from each species was photographed using a photomontage method and compiled into reference sheets to assist with morphological identification. All of the barcodes and images are available on the Mosquitoes of Australia – Victoria (MOAV) project on BOLD, adding southeastern Australia to the Mosquitoes of the World campaign.

Barcoding revealed the presence of *Culex palpalis* within the 113 specimens, which was originally identified as *Cx. annulirostris*, a very similar-looking mosquito and the primary arbovirus vector in Australia. The detection of *Cx. palpalis* is a first for Victoria and highlights the accuracy of the DNA barcoding method. Using COI, all 29 species clustered separately, except for members of the *Cx. pipiens* subgroup, which is a notoriously difficult group to distinguish using standard barcoding regions. The clustering of species was supported by the secondary marker ITS2, which had similar resolving power.

While COI barcoding was achieved using traditional Sanger sequencing (Batovska *et al.*, 2016, DOI: [10.1002/ece3.2095](https://doi.org/10.1002/ece3.2095)), sequencing ITS2 proved to be challenging due to the presence of variable copies. Therefore, high-throughput

sequencing (HTS) was used to sequence ITS2 in the mosquitoes, which allowed the characterisation of all ITS2 variants within individuals (Batovska *et al.*, 2016, *in prep.*). HTS revealed the presence of single nucleotide polymorphisms

(SNPs), microsatellites, and insertions/deletions (indels) in ITS2. The ability to sequence hypervariable markers is one of the benefits of using HTS as a tool for DNA barcoding.

The usefulness of DNA barcoding was recently demonstrated when suspicious mosquito eggs were found in a surveillance trap at the Melbourne International Airport in Victoria, Australia, where *Stegomyia aegypti* mosquitoes had been previously intercepted. Also known as the yellow fever mosquito, *St. aegypti* is responsible for spreading a number of diseases and is exotic to Victoria. Eggs must first be hatched in order to be morphologically identified, which can delay vector control measures and increase the chance of an incursion. The eggs were therefore sent to the VADCP, where they were barcoded and confirmed to be *St. aegypti* within three days. An immediate response followed, and vector control was performed to prevent the establishment of *St. aegypti* in Victoria. This case study demonstrates how DNA barcoding can improve biosecurity and confirms its importance in surveillance programs.

Identifying suspicious mosquito eggs by DNA barcoding can allow the rapid establishment of vector control measures.

A *Stegomyia aegypti* female surrounded by her eggs.



Unraveling the Life Cycle of Shrimp

Written by: Cátia Bartilotti (Instituto Português do Mar e da Atmosfera, Portugal), Joana Salabert (Portugal), and Antonina dos Santos (Instituto Português do Mar e da Atmosfera, Portugal)

The recent rise of the aquarium trade has had a great impact on decapod populations, particularly shrimp and shrimp-like genera, such as *Lysmata* Risso, 1816, *Periclimenes* O.G. Costa, 1844a, *Stenopus* Latreille, 1819 and *Thor* Kingsley, 1878a, most of which show cleaning behaviour and display bright colours. This is especially true for *Thor amboinensis* (De Man, 1888), commonly known as the sexy shrimp for its peculiar behavior. When walking, it raises its tail and moves its body with an exotic elegance, swaying its abdomen back and forth (Debelius, 2001, Crustacea Guide of the World).

As a result of difficulties in linking larval stages of certain marine and estuarine taxa with their adult forms, complete larval development has only been described for three of the 12 species of *Thor*. DNA barcoding, however, can be a useful tool for accurately unraveling the life cycle of a marine species.

Through an integrative taxonomic approach, we aimed to describe the complete larval development of *T. amboinensis*. We first obtained

DNA barcodes of females, and then, based on laboratory-reared animals, we performed a detailed morphological analysis and obtained barcodes throughout larval development. In addition, the barcodes allowed the verification of the recently published hypothesis of the resurrection of the family Thoridae, by providing a species-level perspective of the phylogenetic reconstruction of the relationships within the family as defined by De Grave *et al.* (2014, DOI: [10.1111/zsc.12067](https://doi.org/10.1111/zsc.12067)).

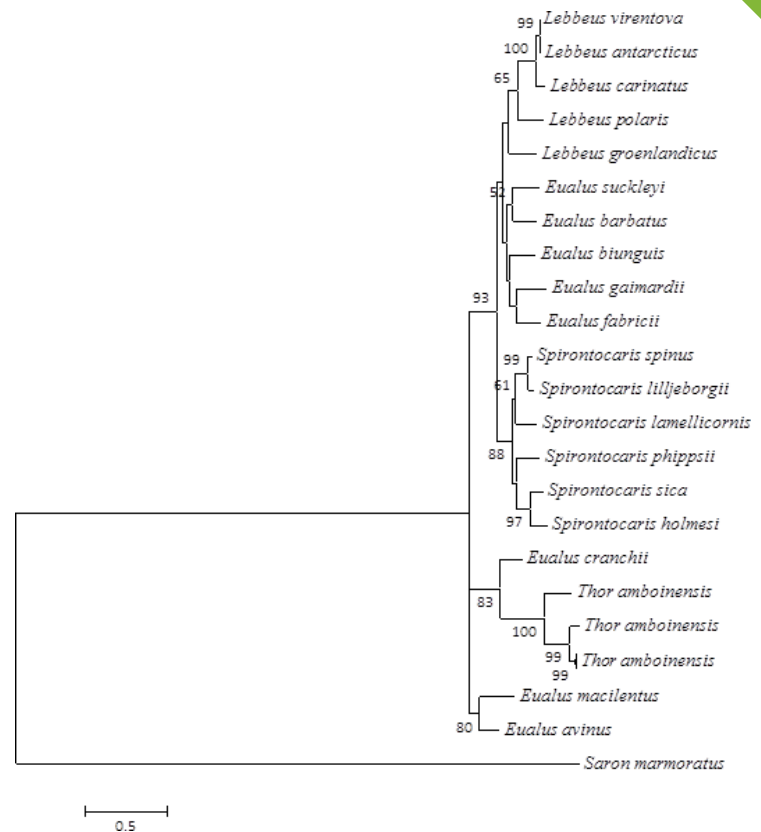
T. amboinensis is a small hippolytid shrimp with a pantropical distribution (Debelius, 2001), living free or associated with corals and anemones. Its larval development consists of eight zoeal stages and one decapodid stage. Within the genus, the number of zoeal stages can vary greatly, from two (*T. dobkini*) to eight (*T. amboinensis* and *T. floridanus*).

“The results of the genetic analysis suggest cryptic speciation for geographically separated populations...”

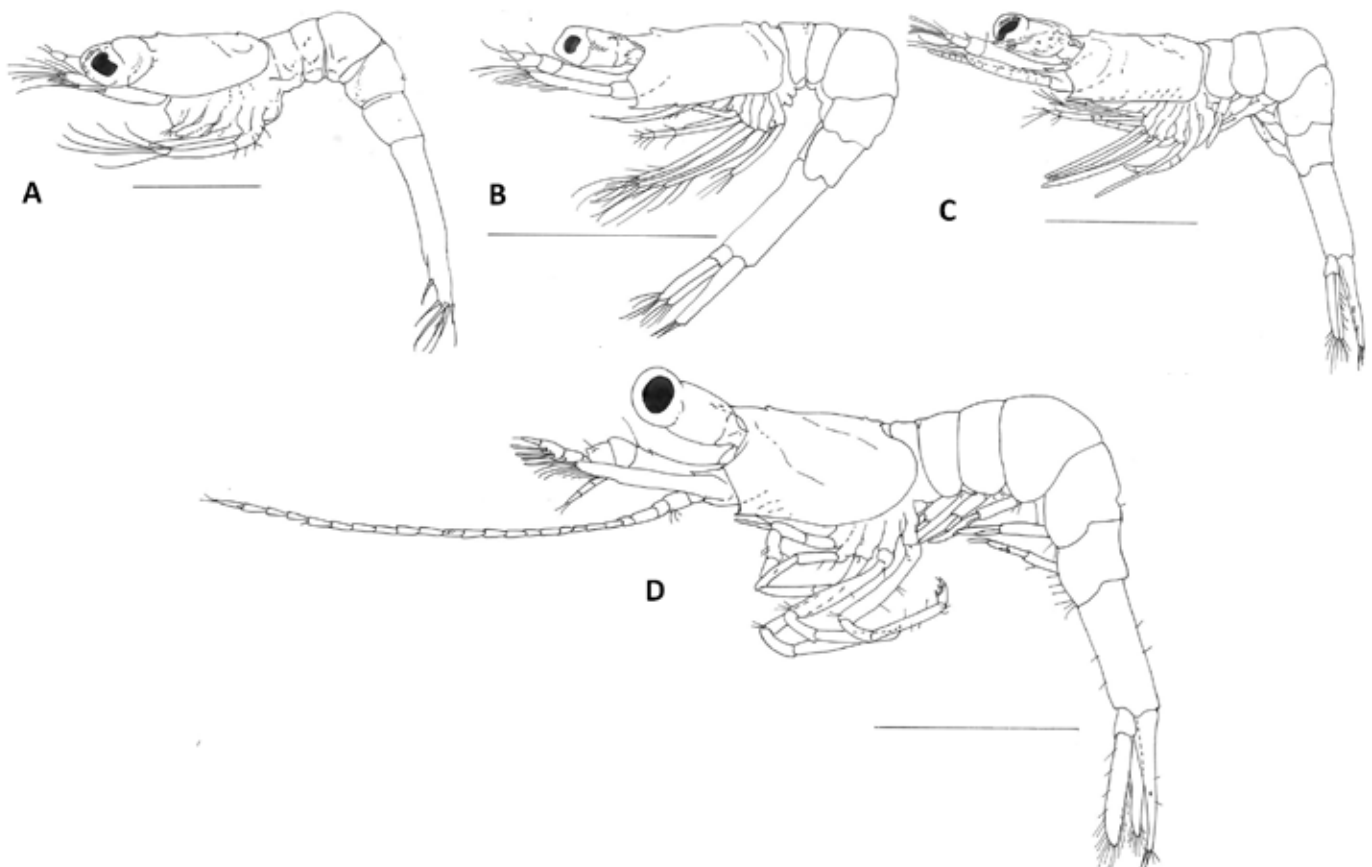
The results of the genetic analysis suggest cryptic speciation for geographically separated populations (Moorea in the French Polynesia, Palmyra Atoll in the Line Islands, and the Philippines), with a minimum distance (p -distance) among populations of 6.8% (between the Philippines and Moorea). In order to clarify the circumtropical species complex of *T. amboinensis*, we suggest the study of the population from the type locality (Amboina, Molucas Sea), using COI as molecular marker. Another interesting result is the paraphyly of the genus *Eualus*, which has been previously noted (e.g. De Grave *et al.*, 2014), and the grouping of *Eualus cranchii* together with *T. amboinensis* with high node support. De Grave *et al.* (2014) suggested a revision of the genus *Eualus* in order to verify the reassignment of *E. cranchii* to a different genus, most likely the genus *Thoralus*, sister taxon of *Thor*.

For more information about the results discussed in this article, see DOI: [10.11646/zootaxa.4066.4.3](https://doi.org/10.11646/zootaxa.4066.4.3)

COI-based phylogenetic tree of representatives of the family Thoridae and one outgroup; only bootstrap values >50% are shown.



Lateral view of the larval stages of *Thor amboinensis*: first (A), fifth (B), and eighth zoeae (C), and decapodid (D). Scale bars represent 0.5 mm (A) and 0.1 mm (B, C, D).



Located mostly along the coast of Brazil, the Atlantic Forest shows a high diversity of landscapes. Among them, the Serra do Mar figures as the longest mountain ridge in eastern South America.

Image credit: Amigos da Estrada da Graciosa

Uncovering Tiger Moth Diversity In Brazil's Most Endangered Biome

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The Atlantic Forest of Brazil is one of the richest and most endangered natural areas of the world and has one of the highest rates of endemism. Located in the most populated region of Brazil, the remaining large fragments are restricted to protected areas, which are under constant threat of being illegally invaded and converted into rural and urban areas. The recent economic growth of Brazil has allowed local research institutions to assess biodiversity patterns in the Atlantic Forest. Our knowledge of insects belonging to the order Lepidoptera has increased considerably since then, but, despite these efforts, a comprehensive inventory remains elusive because of the high diversity of moths.

The subfamily Arctiinae (tiger moths and wooly bears) is a very diverse group with global distribution, though particularly species rich in tropical and subtropical regions. These colorful moths are usually unpalatable and are preyed upon only by highly specialized predators that have to be able to avoid intoxication by alkaloids and other compounds. Many of these moth species are mimetic, resembling butterflies, cockroaches, wasps, and other insects, and many plants depend on them to be pollinated. As part of a PhD project at the Federal University of Paraná, Brazil, a transect ranging from sea level to approximately

1,000 m altitude, located in one of the largest remaining fragments of Atlantic Forest in southern Brazil, was sampled monthly for two consecutive years (2010-2011) using automatic light traps. The initial aim of the project was to record the diversity of tiger moths along an altitudinal gradient and compare the results with other available data. A partnership with the Canadian Centre for DNA Barcoding led to the production of 1,075 DNA barcode sequences, representing most of the diversity sampled among the 14,000 specimens collected during the project.

The moths obtained from the traps were initially sorted into morphologically distinct entities (i.e. morphospecies), a very common approach to assign individuals to putative species when specialized taxonomists are unavailable. Photographs of these morphospecies were sent to two experienced taxonomists for identification. Approximately 50% of the 286 morphospecies were identified to species level, and, after the species boundaries were revised based on the taxonomists' identifications, results were compared to Molecular Operational Taxonomic Units (MOTUs) obtained by DNA barcoding. Interestingly, we found that approximately 30% of our initial morphospecies delimitations did not match the MOTUs derived from DNA barcodes.

“...DNA barcoding allowed us to unveil hidden biodiversity that may be at risk of extinction and that would have otherwise remained undetected. ”

To further investigate these results, research was conducted at the State University of Campinas, also in Brazil, where we dissected specimens and studied the male genital morphology. We then reassigned the specimens to

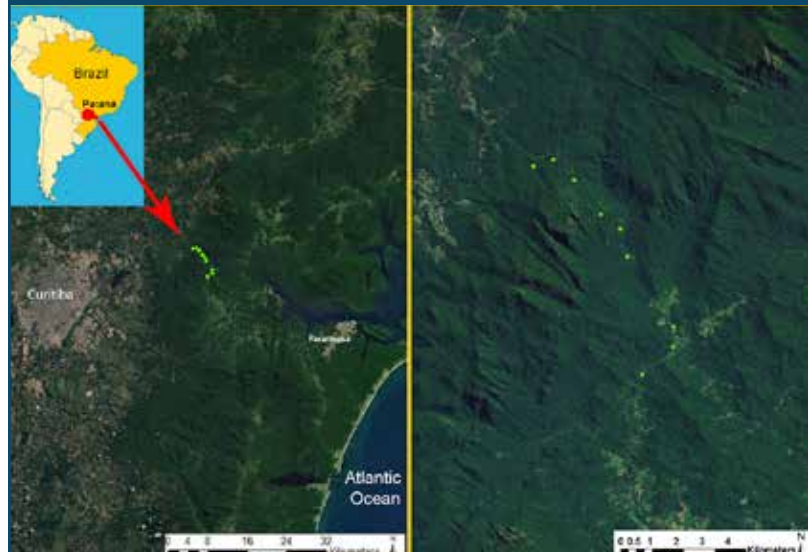
morphospecies accordingly, and we compared such improved morphological species assignments to the MOTUs. It turned out that the new morphospecies corresponded to the MOTUs in 94% of the cases, which means that there was a taxonomic bias of approximately 30% in our species inventory based solely on superficial resemblance among specimens. These results have important implications for different fields such as taxonomy, ecology, and wildlife management because cryptic species (i.e. species that are identical or very similar based on external morphology) may have different biological traits, and thus incorrect species identifications may be detrimental for research and species conservation.

In an area rich in endemic species and severely threatened by human activities, DNA barcoding allowed us to unveil hidden biodiversity that may be at risk of extinction and that would have otherwise remained undetected. Our results certainly apply to other insect groups, many of which are far less documented than tiger moths, and we strongly encourage scientists documenting biodiversity in highly diverse regions of the world to combine traditional morphological taxonomy with DNA barcoding.

For more information about the results discussed in this article, see: DOIs: [10.1007/s10841-015-9753-x](https://doi.org/10.1007/s10841-015-9753-x), [10.1371/journal.pone.0148423](https://doi.org/10.1371/journal.pone.0148423)



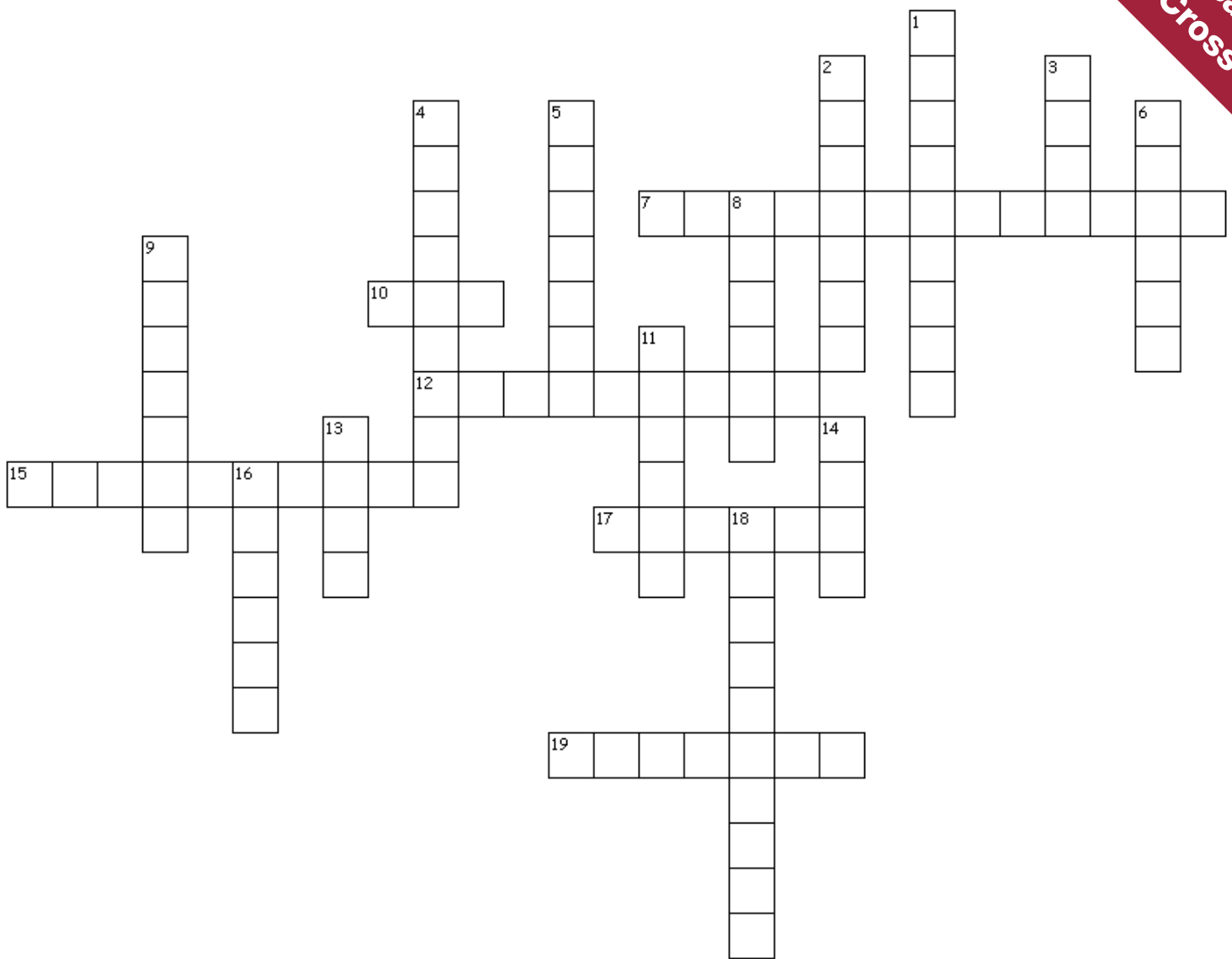
Above: Because of the distance from the equator (c. 29° S) and the relatively high altitude (c. 1,000 m), freezing temperatures can occasionally occur during the winter in the highest part of the sampling region in the Serra do Mar. The picture shows the landscape near one of the sampling sites.



Above: Map depicting the location of the sampling points in the Serra do Mar. The moths were sampled monthly, from January 2010 to December 2011, with automatic light traps.

Below: A species of tiger moth discovered during this study and currently being described as a new species by Dr. Michel Laguerre.





Across

- 7. Massive barcoding using HTS/NGS
- 10. Barcode index number
- 12. Famous butterfly genus in barcode history
- 15. Animal barcode, first word
- 17. Host country of 3rd international conference
- 19. Host city of 5th international conference

Down

- 1. Host country of 4th international conference
- 2. Sequence repository
- 3. Species delimitation method
- 4. Nickname of first seafood barcoding study
- 5. Reference specimen
- 6. Host city of first international conference
- 8. Host city of 2nd international conference
- 9. Initiative to barcode all fish species
- 11. Fungal barcode, third word
- 13. Barcoding work bench
- 14. Largest biodiversity genomics project ever
- 16. Father of DNA barcoding
- 18. Big obstacle of modern taxonomy

***Have you solved the crossword puzzle?
Check your answers in the September
issue of the Barcode Bulletin.***

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 barcodebulletin@gmail.com.