



Barcoding Life Highlights 2013

An illustrated report recently released by Mark Stoeckle (with assistance from Paul Waggoner and Jesse Ausubel) highlights outstanding achievements in DNA barcoding since the 4th International Barcode of Life Conference in Adelaide, Australia, 2011, including scientific advances and new initiatives. In addition to an extensive list of links to DNA barcoding websites that will encourage further collaboration, this report identifies potential future directions for DNA barcoding. The highlighting of certain questions in the report, including 'why does barcoding work?', will most certainly stimulate discussion at the 5th International Barcode of Life Conference in Kunming, China (October 27-31, 2013).

[Download the report \(PDF\)](#)

5th International Barcode of Life Conference

Complete schedule for Kunming



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In addition to the latest DNA barcoding news, this issue of the Barcode Bulletin includes key information for the upcoming conference in Kunming.

5th International Barcode of Life Conference

Welcome letter from the Conference Chair

On behalf of the Organizing Committee, I would like to extend my warmest welcome to you all to the Fifth International Barcode of Life Conference in Kunming, China from 27th to 31st October 2013.

In early 2003, DNA barcoding was proposed by Paul Hebert as a large-scale science to transform our ability to tell the world's species apart, and just a year later, the Consortium for the Barcode of Life (CBOL) was established to promote DNA barcoding activities across the scientific community. The First International Barcode of Life Conference was held in London in 2005, and three conferences followed in Taipei, Mexico City and Adelaide in 2007, 2009 and 2011. The International Barcode of Life project (iBOL), formally launched in fall 2010, is the largest research program ever undertaken in biodiversity science. Progress towards iBOL's key goal of building a barcoding reference library for all species has been rapid. The Barcode of Life Data Systems (BOLD), which plays a central role in assimilating and organizing barcode data, now holds records for more than 2.5M specimens from nearly 200K named species. The international barcode of life community has achieved great things in its first ten years!

Although we celebrate these achievements, there is no room for complacency. Threats to biodiversity represent a massive challenge to us all. The Global Biodiversity Outlook 3 published by UN admitted that we have not met the target agreed by the world's Governments in 2002, 'to achieve by 2010 a significant reduction of the current rate of biodiversity loss at the global, regional and national level as a contribution to poverty alleviation and to the benefit of all life on earth'. In fact, the problem has intensified as a result of global change.

Because of the remarkable progress in sequencing and information technologies, DNA barcoding is well positioned to probe deep ecological and evolutionary questions, and to address environmental and socio-economic issues. For instance, it can inform biodiversity conservation, ecosystem monitoring, forensic investigations, and quarantine programs. Building on the achievements in its first decade, it seems an ideal time for our community to ensure both its commitments and capacity to consolidate the future vision for DNA barcoding into a statement of shared community values, direction and ambition, and to provide a coordinated response from the barcode of life community to the UN Decade of Biodiversity (2011-2020). We have scheduled a session to consider the path forward and to codify our decisions in a formal Kunming Declaration.

Kunming is a beautiful city, the conference venue is excellent, and the line-up of talks, posters, workshops and training courses is first rate. Two sister research institutions of the Chinese Academy of Sciences, Kunming Institute of Botany and Kunming Institute of Zoology, are delighted to be hosting the fifth conference. Our meeting in Kunming has attracted more than 400 delegates from 43 nations sustaining this tradition of true internationalism. My colleagues and I thank you for taking the time from busy schedules to join us here for a meeting that we hope you will both enjoy and find scientifically stimulating.

Enjoy your participation at the meeting and your stay in Kunming.

De-Zhu Li, Conference Chair



5th International Barcode of Life Conference

Plenary discussion on the formation of a DNA barcoding society scheduled for October 28th

DNA barcoding has become an internationally recognized methodology in biodiversity science and is finding its way into a wide range of socially beneficial practical applications. Barcoding also exemplifies the ability of scientists to self-organize and collaborate nationally, regionally and internationally. With the Fifth International meeting representing a decade of international cooperation and achievement, it is time to consider options for organizing a professional society solely devoted to DNA barcoding and its scientific, technological and socio-economic dimensions. As with the formation of other international societies, there are many factors to consider. Presuming the need for a professional society is accepted, its scope must be delineated.

Other considerations include how best to promote scientific and technical excellence in biodiversity science at a planetary level, what the best teaching and outreach opportunities are, how to prioritize applications of barcoding to maximize socio-economic impact, and how to integrate barcoding into the complex international landscape of species protection and access and benefits sharing. Practical considerations for any new society also include its policies and procedures, communications and publications, and conference organization. A session at the conference in Kunming will introduce these topics via a panel presentation and a moderated discussion.

Complete conference schedule

General agenda, plenary sessions, and parallel sessions for October 27th-31st, 2013

General agenda					
	Sunday, 10/27	Monday, 10/28	Tuesday, 10/29	Wednesday, 10/30	Thursday, 10/31
Morning	Preconference Events - BOLD Update, Discussion Session on Sequencing Methods (Next Generation)	Opening Ceremony			
		1st Plenary Session	3rd Plenary Session	5th Plenary Session	7th Plenary Session
		2nd Plenary Session	4th Plenary Session	6th Plenary Session	8th Plenary Session
Lunch		Lunch	Lunch	Lunch	Lunch
Afternoon		1st Parallel Session	Free Afternoon & Excursions	3rd Parallel Session	5th Parallel Session
		2nd Parallel Session		4th Parallel Session	
Evening	Welcome Reception Dinner at Lian-Yun Hotel	Plenary Discussion		Poster Session & Beverages	Closing Remarks/ Cocktail Hour at Kunming Institute of Botany

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Complete conference schedule-

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Plenary sessions			
Monday, 10/28	Tuesday, 10/29	Wednesday, 10/30	Thursday, 10/31
Moderator: Bob Hanner	Moderator: Laurence Packer	Moderator: Wen-Ying Zhuang	Moderator: De-Zhu Li
1st Plenary Session: iBOL Update	3rd Plenary Session: Implications for Biodiversity II	5th Plenary Session: Ecological Implications	7th Plenary Session: Societal Implications
1 De-Zhu Li	7 Bob Murphy	13 Graham Stone	19 David Schindel
2 Gerhard Haszprunar	8 Alfried Vogler	14 Beth Clare	20 Linda Santschi
3 Paul Hebert	9 Michael Balke	15 Tomas Roslin	21 Tania Bubela
2nd Plenary Session: Implications for Biodiversity I	4th Plenary Session: Implications for Biodiversity III	6th Plenary Session: Ecosystem Implications	8th Plenary Session: Final Reflections
4 Pete Hollingsworth	10 Jan Pawlowski	16 Mehrdad Hajibabaei	22 Da-Wei Huang
5 Michelle Van der Bank	11 Gary Saunders	17 Douglas Yu	23 John Kress
6 Dario Lijtmaer	12 Zhu-Liang Yang	18 Bao-Li Zhu	24 Richard Lane

Parallel sessions - Monday, 10/28					
1st Parallel	Plants I <i>Moderators: Xue-Jun Ge, John Kress</i>	Fishes I <i>Moderator: Claudio de Oliveira</i>	Insects I <i>Moderator: Michael Balke</i>	Education <i>Moderator: Dirk Steinke</i>	Next Generation <i>Moderator: Douglas Yu</i>
	1 Natasha de Vere 2 Juan Liu 3 Kevin Burgess 4 Yu Song 5 Nancai Pei 6 Zhe-Chen Qi 7 Lian-Ming Gao	1 Bob Hanner 2 Monica Mwale 3 Thomas Kneibelsberger 4 Lourdes Vasquez Yeomans 5 Rajiv Ravi 6 Gontran Sonet 7 Claudio De Oliveira	1 Qing-Hua Liu 2 Cecilia Kopuchian 3 Sean Prosser 4 Uraiwan Arunyawat 5 Mikko Pentinsaari 6 Marko Mutanen 7 Fan Jiang	1 Dirk Steinke 2 Janis Geary 3 Thibaud Decaens 4 Amanda Naaum 5 Torbjorn Ekrem 6 David Castle	1 Xin Zhou 2 Yin-Qiu Ji 3 Eric Coissac 4 Shadi Shokralla 5 Catharine Bruce
BREAK					
2nd Parallel	Plants II <i>Moderators: Jie Li, Michelle van der Bank</i>	Pollinators <i>Moderators: Laurence Packer, Hong Wang</i>	Data Analysis <i>Moderator: Ian Hogg</i>	Environmental Monitoring <i>Moderator: Xin Zhou</i>	Amphibians & Reptiles <i>Moderators: Jing Che, Andrew Crawford</i>
	1 Oluwatoyin Ogundipe 2 Wen-Bin Yu 3 Tao Cheng 4 Ahmed Gawhari 5 Xue-Wei Jiang	1 Mark Stevens 2 Laurence Packer 3 Osamu Tadauchi 4 Scott Groom 5 Rebecca Dew	1 Wei Zhang 2 Long Fan 3 Sujeewan Ratnasingham 4 Mari Kekkonen 5 Emanuel Weitschek	1 Hsuan-Wien Chen 2 Natasha Serrao 3 Jeremy deWaard 4 Wen-Hui Song 5 Sandi Willows-Munro	1 Ngoc-sang Nguyen 2 Andrew Crawford 3 Jing Che 4 Mariana Lyra 5 Zoltan Nagy

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Complete conference schedule-

Continued from page 4

Parallel sessions - Wednesday, 10/30					
3rd Parallel	Informatics <i>Moderator: Jun-Cai Ma</i>	Medicinal Plants I <i>Moderators: Shi-Lin Chen, Natasha de Vere</i>	Marine Barcoding <i>Moderator: Michael Rapauch</i>	Pests, Parasites, Etc. <i>Moderator: Virginia León Rêgagnon</i>	Insects II <i>Moderators: Axel Hausmann, Qiao-Yun Yue</i>
	1 Lin-Chun Shi 2 Li Liu 3 Sujeevan Ratnasingham 4 Vincent Robert 5 Douglas Chesters 6 Chang Liu 7 Taryn Athey	1 Shi-Lin Chen 2 Lu-Qi Huang 3 Allan Showalter 4 Melanie Schori 5 Pang-Chui Shaw 6 Jyoti Maharjan 7 Qing-Jun Yuan	1 Hong Zhou 2 Adriana Radulovici 3 Filipe Costa 4 Hsi-Nien Chen 5 Michael Raupach 6 Joong-Ki Park 7 Sergio Hernandez-Trujillo	1 Bob Hanner 2 Sean Locke 3 Virginia León Rêgagnon 4 Amanda Naaum 5 Xin Zhou 6 Gontran Sonet 7 Hua-Rong Zhang	1 Rui Chen 2 Alejandro Zaldivar-Riveron 3 Xiao-Ye Li 4 T. Fatima Mitterboeck 5 Carlos Lopez-Vaamonde 6 Axel Hausmann 7 Pablo Damian Lavinia Oblanca
BREAK					
4th Parallel	Other Invertebrates <i>Moderator: Manuel Elías-Gutiérrez</i>	Fishes II <i>Moderator: Bob Hanner</i>	Plants III <i>Moderators: Lian-Ming Gao, Andy Lowe</i>	Enviromental Barcoding <i>Moderator: Evgeny Zakharov</i>	Insects III <i>Moderator: Scott Miller</i>
	1 Frank Stokvis 2 Ian Hogg 3 Mark Stevens 4 Manuel Elías-Gutiérrez 5 Pavel Stoev	1 Natasha Serrao 2 Wazir Lakra 3 Matthias Geiger 4 Siti Azizah Mohd Nor 5 Subrata Trivedi	1 Subramanyam Ragupathy 2 Ting-Shuang Yi 3 Rachel Acil 4 Sangita Shrestha 5 Kyeonghee Kim	1 Shadi Shokralla 2 Min Tang 3 Aurelie Bonin 4 Chen-Xue Yang 5 Ian Hogg	1 Rodolph Rougerie 2 Paul Hebert 3 John James Wilson 4 Scott Miller

Parallel sessions - Thursday, 10/31				
5th Parallel	Plant Methods <i>Moderators: Pete Hollingsworth, Shi-Liang Zhou</i>	Fungi & Algae <i>Moderator: Jian-Ping Xu</i>	International Collaboration <i>Moderator: David Castle</i>	Medicinal Plants II <i>Moderators: Lu-Qi Huang, Subramanyam Ragupathy</i>
	1 Anna Williams 2 Shi-Liang Zhou 3 Xi-Wen Li 4 Chang-Hao Li 5 Jeffrey Boutain 6 Jeff Bennetzen	1 Myung Sook Kim 2 Helena Korpelainen 3 Chun-Yan Yang 4 Irinyi Laszlo Miklos 5 Benjamin Stielow 6 Thorsten Lumbsch	1 Olivier Maurin 2 Axel Hausmann 3 Pablo Tubaro 4 Cecilia Kopuchian 5 Alex Borisenko 6 Manuel Elías-Gutiérrez 7 Mailyn Gonzalez	1 Zhi Chao 2 Haruka Asahina 3 Sathishkumar Ramalingam 4 Neesha Rana

New and Noteworthy:

Latest developments in the world of DNA barcoding

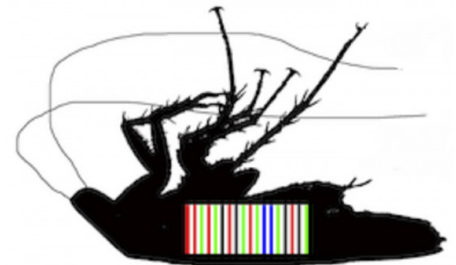
The Zoological Museum Alexander Koenig in Bonn, Germany has received the award 'Ort des Fortschritts' (site of progress) by the North Rhine-Westphalian research ministry. This award recognizes the work of the Leibniz Institute for Animal Biodiversity especially for coordinating and leading the German Barcode of Life Project (GBOL).



Barcoding Fauna Bavarica will receive another Euro 750 000 from the Bavarian State Ministry for Science, Research and the Arts for five more years work on the project. The funds will help to fill gaps in the current library by including fungi, lichens, algae, diptera, red list species, FFH species (Fauna-Flora-Habitat directive of the EU), parasites, and neozoans.



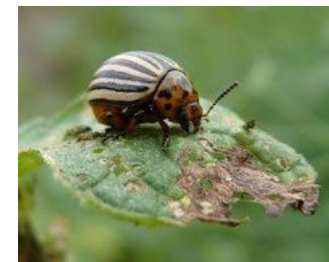
First preliminary results from the National Cockroach Project indicate that cockroaches in certain city neighborhoods of New York share the same genetic makeup -- and they differ from roaches in their neighboring hoods. The National Cockroach Project, spearheaded by Mark Stoeckle, Rockefeller University, New York, started in December 2012 and involves citizen scientists across the US to sample cockroaches and send them in for barcode sequencing.



Barcoding P.A.T.H.S. (Plant & Algal Type & Historical Specimens) is a new library of reference sequences, easy to update, and against which putative new species or other plant material can be compared. As the acronym P.A.T.H.S. suggests, specimens comprise water, and land plants and algae, interpreted sensu lato to include not only microalgae and seaweeds, but also cyanobacteria. The project focuses solely on reference specimens and includes type specimens and historical material.



Earlier in the year PestBOL, a new DNA barcoding resource, went live. The Plant Pest Barcoding site provides summary information on DNA barcode coverage for invertebrate pests of significance to global plant production. It is intended for use by the plant protection community, including regulators, researchers, and growers. By highlighting gaps in coverage, it also supports coordinated efforts to further the development of DNA Barcode reference libraries for pest arthropods.



Education and Barcode of Life (eBOL):

Community Web Portal launched in February 2013 (www.educationandbarcoding.org)

The new campaign aims to expand the community of iBOL data contributors and enhance bioliteracy

In March 2013, BOLD surpassed the 2M sequence mark – a significant milestone in iBOL's goal to assemble 5M reference barcode records by the end of 2015. Ongoing efforts to achieve this ambitious goal will be aided by the Education & Barcode of Life (eBOL) project, a pioneering campaign that is currently being led by California-based Coastal Marine Biolabs (CMB) and the Biodiversity Institute of Ontario (BIO). Unlike other barcoding campaigns that focus their activities on particular taxa or geographical regions, eBOL's primary efforts are aimed at empowering a global community of prospective student data contributors.

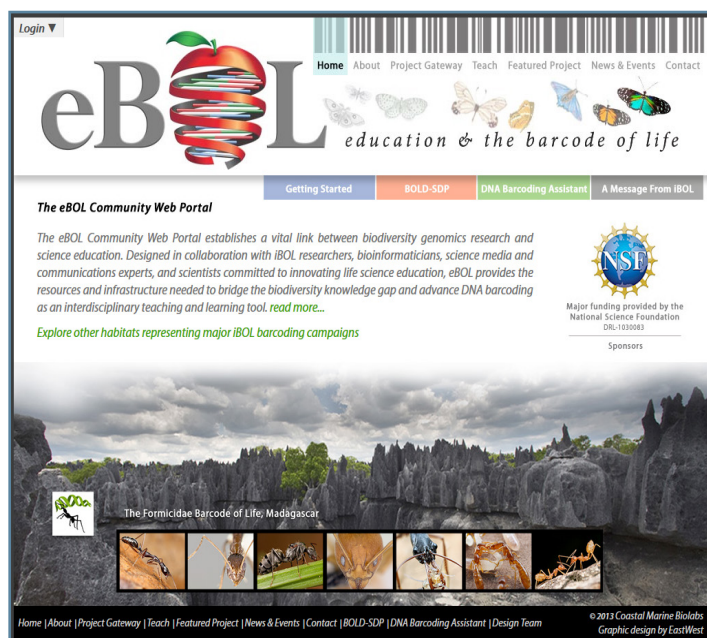
In the United States alone, there are nearly 30,000 secondary schools serving over 16M students, and some 4,000 degree-granting colleges and universities with a combined undergraduate enrollment of over 14M students. The widespread introduction of DNA barcoding technology into high school and undergraduate science labs around the globe therefore



holds enormous potential to significantly expand the worldwide community of iBOL contributors while at the same time advancing a new strategy to innovate and update life science curricula.

The major challenge in serving these compound interests rests in providing a new community of iBOL contributors with access to high quality technology resources that not only foster a deep understanding of the concepts and methods of DNA barcoding, but that enable them successfully to collect, generate, and share scientifically relevant data that comply with current barcode data standards. To address these challenges, eBOL assembled a team of scientists, informaticians, and science media experts to create the eBOL Community Web Portal, which was formally launched in February 2013 with major support from the National Science Foundation, the Ontario Ministry of Research and Innovation, and Genome Canada.

At the center of this new open-access resource is the BOLD Student Data Portal (BOLD-SDP), a customized student interface to the BOLD researcher workbench that enables students and instructors to manage, assemble, analyze, and share reference barcode data through simplified consoles and intuitive workflows. A carefully conceived, 3-tier validation system ensures that data standards are met before records are transferred to the BOLD reference library and published in INSDC.



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Education and Barcode of Life (eBOL)-

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In addition to this centralized student workbench, the eBOL community web portal integrates mobile computing technology for students to capture specimen metadata in the field, an expansive digital media library for instructors to teach the conceptual and technical aspects of DNA barcoding, and a project gateway that provides a web-based forum for scientific and educational groups to exchange ideas and information that advance DNA barcoding as a teaching and learning tool.

Although the creation of the eBOL Community Web Portal represents an important first step toward the broad-scale engagement of students in the iBOL mission, the long-term success of this effort will be critically reliant upon the sustained involvement of

the scientific community in bridging a number of additional resource gaps. We therefore call upon our iBOL colleagues to assist our team in its efforts to provide students with access to tissue from curated specimens, to supervise new specimen collections, and to develop new educational resources for the benefit of the eBOL community. At a time when unprecedented numbers of scientists seek new strategies to broaden the impact of their research endeavors, involvement in these activities may establish a new collaborative outreach model to encourage and/or deepen the participation of the scientific community in enhancing secondary and post-secondary life science education.

Written by: Ralph Imondi

The School Malaise Trap Program:

Involving students in active science through the exploration of insect diversity

The ultimate objective of the School Malaise Trap Program is to engage and inspire the next generation of youth in communities across southern Ontario, and ultimately North America, to become scientists, environmental stewards, and otherwise ecologically-minded citizens. The project aims to address and enhance the teaching of Canadian biodiversity and environmental stewardship in primary and secondary schools by providing an exciting, hands-on, technologically savvy, and scientifically relevant educational experience.

The School Malaise Trap Program engages primary and secondary school students and teachers in exploring insect diversity in their schoolyards through DNA barcoding. Each school, or classroom, receives a Malaise Trap for collecting specimens over a select time frame (~1-2 weeks) with a goal of sequencing

about two-hundred arthropod specimens from each trap.

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The School Malaise Trap Program-

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The 2011 pilot of the program that involved three high schools led to the recovery of 230 species, 19 of which were new to the Barcode of Life Database (BOLD). In spring 2013, the program involved 60 schools and led to the recovery of 1,392 species, 276 of which were new to BOLD. Through this hands-on program, students and teachers are provided with a real sense of discovery, as well as scientific merit by contributing valuable data to the International Barcode of Life project.

The School Malaise Trap Program is broken down into four core components: 1. Supplementary teaching material, 2. BIObus school visit, 3. Malaise trap deployment, 4. Collection results. The program is currently targeted at Grades 6 and 12 in compliment to these levels' biodiversity and molecular genetics units (Ontario curriculum) respectively. Falling in-line with provincial teaching objectives, each grade level is provided with access to supplementary teaching materials, i.e. lesson plans and activities, which compliment the program. These teaching resources are made available online on the program's webpage (<http://malaiseprogram.ca>). Furthermore, schools are visited by the BIObus, BIO's field research vehicle. During this visit, an interactive talk is delivered at the school followed by a tour of the BIObus. Students are introduced to concepts of biodiversity and DNA barcoding, as well as to the life of a field biologist. As part of the tour, program participants and BIO staff can interact one-on-one. Students and teachers are invited onboard the BIObus, insects collected in Canada are displayed, arthropod trapping field methods are discussed, and staff reminisce and answer questions about BIObus collection expeditions.

At the time of the BIObus school visit, classrooms are equipped with their insect collecting packages, including a Malaise Trap and instructions on deployment. All schools are instructed to deploy their traps during a pre-determined time frame, enforcing

a standardized protocol to allow for data comparison as well as to ensure timely delivery of results. At the end of the collection period all specimens are picked up and taken to BIO where they are analyzed and barcoded.

In conclusion of the program, trap results are emailed to teachers, along with tools for teachers to present these results to their classes. In the results package, classrooms are provided with individualized reports that provide information such as total specimen numbers, breakdown of major insect groups caught, species number with full list and images, biodiversity ranking in comparison to other participating schools, and indication of their contribution to the Barcode of Life database. Furthermore, a full program report gives an overview of data obtained across all the participating schools and traps, including information on the scope of participants, ranking results for different biodiversity measures, interesting specimen discoveries, and much more.

The effectiveness of the program is continuously gauged based on the response from teachers, students and school administrators of the participating schools. Surveys are distributed to teachers and school administrators along with each school's final program reports. This feedback is invaluable for making improvements to the project.

The School Malaise Trap Program is more than just a hands-on learning experience. It directly involves students in real and active science. It also provides an opportunity for students to be citizen scientists and contribute to a global project of discovery, iBOL, which is opening doors to plethora of practical applications.

Written by: Dirk Steinke

San Diego Biodiversity Project:

Engaging students and promoting biodiversity

In the last year, over a thousand undergraduate students at the University of California, San Diego became barcoders. Human biology majors, physiology majors, students that have never (willingly) touched a bug or thought about biodiversity, all participated in a project to create a species inventory of invertebrate animals at a reserve adjacent to campus.

The San Diego Biodiversity Project is a US National Science Foundation-funded project to incorporate authentic research into the biology curriculum. Students generate novel information - species barcodes - which they will communicate to the larger research community through the BOLD database.

San Diego is a hotspot of biodiversity with many endemic, as well as many imperiled, species. The immediate scientific goal of the project is to barcode the poorly known invertebrates at the Scripps Coastal Reserve, a 350 hectare refuge that is part of the University of California Natural Reserve System. The small reserve is surrounded by houses but despite the urban location there is plenty of work to be done to document the biodiversity. Last fall students collected spiders in the first week of class and found that less than 20% had good species matches in the BOLD database. In previous quarters students determined the species status of a morphologically variable polychaete in the sandy beach and they worked on developing a barcode marker for Africanized honey bees.

The pedagogical goal is to engage students with a research experience. Much has been written about the benefits of research for undergraduate science students, but this usually takes the form of a summer internship for a couple of students in a research lab, or an advanced lab or honors course with a small enrollment. Each quarter the San Diego Biodiversity Project is able to offer 200-300 students this research experience through collaboration. Students in ecology labs collect insects and other invertebrates in the field, document ecological information, and take high-resolution photographs. They then pass the specimens to students in the molecular biology lab courses, and these students generate the barcode sequence data. All of this information is uploaded onto the project database, where all of the students can see the results of their work and that of their colleagues. In addition, student-generated data are used elsewhere in the Biology curriculum to teach how the mutational process works and how the history of diversification is written in DNA sequences.

By the end of the course the undergraduates had a better understanding of the process of science, they felt more confident about doing research, and they had a heightened awareness of their own ability to contribute new knowledge to the field. They also had a greater resolve to help preserve biodiversity. One student summed up much of the feedback, "I like doing a project that counts for something outside of the class."

Written by: Heather Henter



Images credit: Heather Henter

Who Ray?!

DNA barcoding unveils the diversity of skate species sold in 'ray' products

The deliberate act of falsely representing, labelling or advertising food, known as “food fraud”, is not a recent phenomenon. The deceitful adulteration of food has a long history, based on the promise of making a quick profit through dishonest customer transactions. As such, it has generally been associated with economic gains through the substitution of a cheaper more abundant species, for a more highly prized one.

The uncovering of the widespread horse meat scandal across Europe indicates how pervasive, and often unnoticed, this problem can be. Whilst it is difficult, if not impossible, to estimate just how widespread food fraud has been in the past, DNA barcoding provides a robust solution to identifying species sold to us in fresh foods – including seafood.

The problem of substitution and mislabelling of seafood raises the potential for economic fraud and human health issues (mis-selling dangerous goods and/or allergens). It also raises a serious conservation problem; mislabelling could provide a route for prohibited species (that are

illegally landed) to be sold, and it removes the power of consumers to avoid vulnerable or threatened species through responsible purchasing.

Ray is the common term under which a number of different species of skates fished from the Northeast Atlantic are commonly sold in Western Europe. Unlike the typically consumed white fish, skates are a type of cartilaginous fish more closely related to sharks, which fall under the same subclass, *Elasmobranchii*. “Ray” are also traditionally sold as ‘wings’, where the body and skin are removed (leaving the large pectoral fins for sale), making morphological identification impossible.

The conservation status of skate varies by species, with the European Union now prohibiting landings of the species that have undergone very steep declines in abundance. Therefore, it’s difficult for consumers to make responsible decisions when purchasing fish labelled as “ray”. In our recently published study, we found that a minimum of six different species are currently being sold under the term “ray” in the United Kingdom and Republic of Ireland.

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Image credit: Andrew Mark Griffiths

Who Ray?! -

Continued from page 11



Image credit: Andrew Mark Griffiths

The great news for skate conservation was that none of these were among the severely declining, prohibited species; probably reflecting the current scarcity of these species and strong efforts of fishermen, stakeholder and conservation groups to avoid landing them.

Despite this, three of the species identified (blonde ray, thornback ray and shagreen ray), all grow to a large size (>1m) making them potentially vulnerable to over-fishing, and are included in the “near threatened” category within the IUCN Red List (which lists threatened species of plants and animals). In particular, the blonde ray was the most commonly identified species, but it has been awarded the lowest sustainability rating by the Marine Conservation Society’s Good Fish Guide, suggesting consumers should avoid purchasing them.

In the UK and Irish markets, very few products sold as “ray” came with any further description that identified the species. Although the use of common or umbrella terms in the labelling of seafood is currently legal within the EU, more descriptive labels that identify the species of fish being sold would make it easier for shoppers to make responsible decisions when purchasing fish.

What was concerning about this investigation was that, of the very few fish sold as “ray” that were

packaged with detailed information to the species level, one third were found to be incorrectly labelled (two out of six samples). While packaging indicated the fish were species of lower conservation concern, through DNA analysis, these products were identified as thornback ray, a near threatened species.

As the world’s human population grows and food production systems become larger and more mechanised, we are becoming increasingly separated from the original source of our food. Beyond trusting product labels, it’s difficult to have confidence in knowing what exactly we are eating and where it has originated from. Particularly in the seafood industry, where species scarcity is of direct environmental concern, labels should contain adequate and accurate information that can enable consumers to make responsible choices, if this is important to them.

The work was conducted at The University of Salford (UK) and University College Dublin (ROI) and was part funded by the European Union’s InterReg “LabelFish” Project

Written by: Andrew Mark Griffiths, Dana Miller, and Stefano Mariani

Preserving DNA for Successful Barcoding:

Recent advances in storage methods

Preservation of DNA samples during storage is crucial for DNA barcoding as degradation decreases the probability of successfully obtaining the barcode of a sample. In the past, this preservation has been achieved, even over many years, by storing samples in freezers at -20°C . However, these low temperature requirements become problematic both for the shipment of samples between facilities and for long-term storage, where the potential for freezer failure exists.

Recently, it has been suggested that dry storage at room temperature may be possible with the addition of a preserving agent. Natalia Ivanova (pictured on right) and Masha Kuzmina at the Biodiversity Institute of Ontario tested the impact of various factors on rates of degradation in insect DNA extracts, including the effectiveness of multiple preserving agents, to provide recommendations for the short-term and long-term storage of DNA samples.

The amount of handling during dry storage was one of the factors considered in their study, with greater handling contributing to higher rates of DNA degradation likely as a result of fluctuations in humidity levels and increased exposure of the DNA to air. A second factor affecting DNA preservation was the concentration of the DNA sample. Concentrated samples showed high PCR success, represented by successful amplification of the COI barcode region, in both their short-term (up to four months) and long-term (up to four years) storage experiments. Diluted samples without any preserving agent degraded completely in just two months at room temperature compared to two years for the degradation of concentrated samples.

The addition of a preserving agent to dry DNA samples played a major role in obtaining successful amplification of the barcode region following storage at room temperature or higher temperatures. The

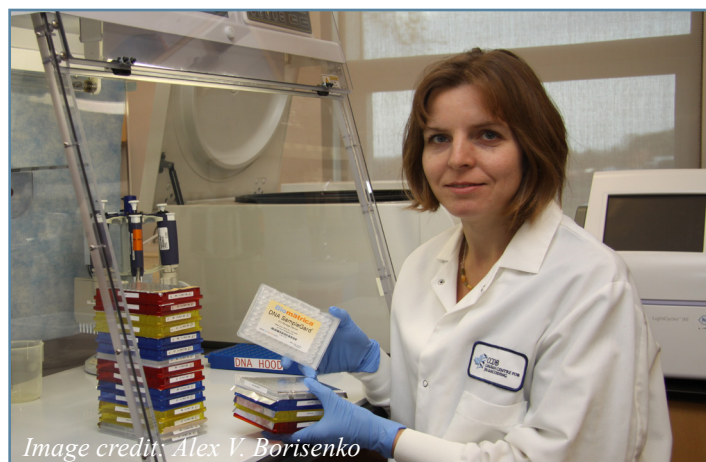


Image credit: Alex V. Borisenko

commercially-available Biomatrixa was generally the best preserving agent compared to the handmade polyvinyl alcohol (PVA) and trehalose. Interestingly, high temperatures during storage affected the reliability of PVA and trehalose in different ways, with trehalose-protected samples showing higher PCR success when stored at 56°C compared to room temperature and the opposite outcome being observed for DNA samples with PVA added.

Based on these results, the authors recommend the addition of a preserving agent to prevent DNA degradation for short-term dry storage at room temperature, which would allow for easier shipments of DNA samples. However, for long-term storage, samples that were frozen maintained higher DNA quality after four years, as demonstrated by longer sequences and a stronger signal, than dry samples kept at room temperature with a preserving agent. They therefore conclude that storage at -20°C remains the most reliable option for samples intended for DNA barcoding.

Written by: Emily Berzitis

Barcoding a Natural History Collection:

An ideal resource for the development of a comprehensive DNA barcode reference library

Barcoding natural history collections is critical for the construction of a DNA barcode reference library, particularly in cases of rare or extirpated species where the museum voucher may represent the only available source of genetic material. Unfortunately, large scale studies of this nature are lacking due to the inherent degradation of DNA in many museum specimens. We therefore initiated a barcoding “blitz” in 2011 that involved the data digitization and molecular analysis of over 40,000 specimens of Lepidoptera in the Australian National Insect Collection in Canberra, ACT. In addition to taxonomic information, data digitization also included metrics such as specimen age, locality information, and original collector. Total body weight was also measured for select species using vouchers archived at the Biodiversity Institute of Ontario. These data, combined with barcoding results, revealed some interesting findings pertaining to the likelihood of obtaining DNA sequences from museum specimens.



While it is generally known that the older the specimen, the less DNA available for sequencing, results from this study showed that sequence recovery declines rapidly in the first 30 years, then holds more or less stable for the next 30 years before dropping to very low levels after 60 years of age (Figure 1). Even more interesting is the finding that factors other than age can have a significant effect on sequencing success. Total body weight does not have an effect on young specimens - barcodes were recovered from small species just as easily as large species, as long as they were under 10 years old. However, the older the specimen, the more size matters, with the DNA of larger species persisting for a longer period of time. Perhaps the most surprising result was the degree to which the original collector affected modern day attempts to recover DNA from their specimens. DNA sequences were very difficult to recover from the specimens of certain collectors, regardless of specimen age, size, or taxonomy. This “collector effect” - the ability of the original collector to significantly affect barcoding success - is likely due to the method in which the collector killed and/or preserved their specimens. Specimens killed and preserved with chemicals that damage DNA will be difficult to barcode regardless of their age, size, or taxonomy. Overall, sequence data were recovered from 86% of the specimens and a compliant barcode (>487 bp) was recovered from almost all species included in this study.

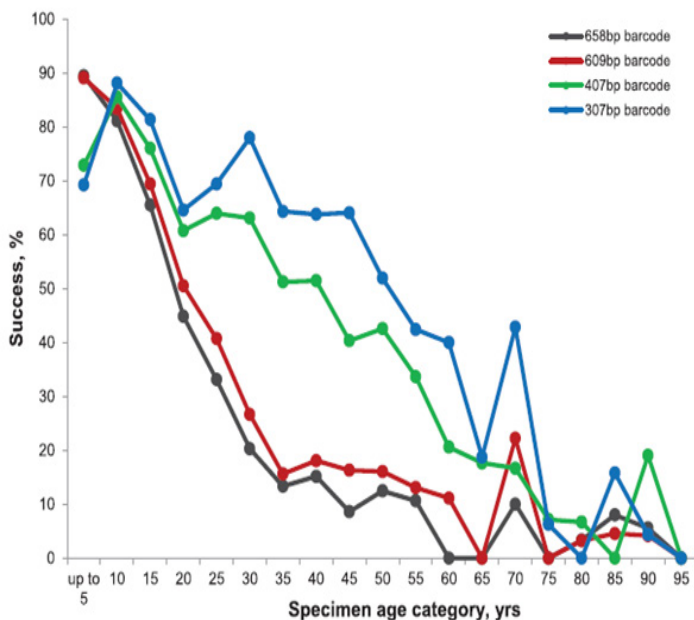


Figure 1. Percent success of DNA barcode recovery for four different primer sets (targeting four different sequence lengths) compared to specimen age.

Written by: Sean Prosser and Jayme Sones

Turbotaxonomy:

Integrating barcoding, imaging and morphology

Thailand is a country of great biodiversity despite having intensive and expansive agriculture, with 90 terrestrial national parks totaling more than 48,000 km² plus many more forest reserves. However, apart from some charismatic groups, a lot of its invertebrate fauna is poorly known, and of the insects, the parasitic Hymenoptera are probably one of the least well studied groups. The study described here was facilitated by the TIGER (Thailand Inventory Group for Entomological Research) program which sampled insects in 25 national parks using Malaise traps from 2006 to 2008 (see <http://sharkeylab.org/tiger>).

Recently we have been working largely on the braconid wasp subfamily Rogadinae that are particularly useful in terms of study of host relationships because all species pupate within the mummified skin of their caterpillar host. This means that if specimens are preserved along with the mummy from which they emerged, the parasitoid's host can be verified to veracious taxonomic levels and may well be suitable for DNA barcoding too.

The cosmopolitan genus *Aleiodes* dominates the Rogadinae, and only the Holarctic fauna has received a reasonable amount of taxonomic attention, and even in the Western Palearctic, morphological identification is often difficult (and can be impossible) and beset with species complexes and cryptic species. Moving to the tropics, the diversity becomes staggering, though less so in the Neotropics where it appears to be functionally largely replaced by *Triraphis*, a related genus from another tribe. The vast majority of species in Africa and Southeast Asia are undescribed and there are many species groups where morphological separation without ancillary knowledge is largely guesswork. Indeed surprisingly few tropical species have been described which is a big aid in trying to treat such a group taxonomically as it means that relatively little effort has to go into



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trying to recognize species based on old museum type specimens.

Barcoding specimens from both the TIGER program samples and our own collecting, from all over the country, suggested there were more than 150 species present. Some specimens that failed to yield sequences were clearly morphologically different and in addition, more putative morphospecies were present in the accessions collection of the Natural History Museum in London, mostly about 20-50 years old. We therefore decided to try to integrate both the sequenced (i.e. *Aleiodes* (*Arcaleiodes*) *siamensis* Quicke & Butcher, pictured above) and unsequenced (i.e. *Aleiodes* (*Arc.*) *vanachterbergi* Quicke & Butcher, pictured below) material in a revision, which meant providing an identification key to all those we recognized as well as formal taxonomic descriptions.

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Turbotaxonomy -

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With 182 species in total, of which 179 appeared to be new to science, this task was quite daunting. Even coming up with so many new names was not trivial, and a lot of use was made of characters from Sir Terry Pratchett's Discworld stories and one whose diagnostic sequence of barcode bases was G, A, G, A lent itself well to being named after Lady Gaga (*Aleiodes gaga*) which received a fair amount of publicity.

Clearly, the amount of work that would have been involved presenting the normal, in depth type of description of traditional braconid taxonomy, with many measurements and verbose characterizations of sculpture would have meant a very long piece of research. Instead we chose to see whether a faster approach was possible, relying largely on montages of stacked colour photographs of more or less standardized views of each species supplemented with fairly short text descriptions of features of likely importance that could not be discerned readily from most of the photographs. We termed this approach 'turbo-taxonomy'.

One of the particular, and unsolved, challenges posed by this work involved an apparently very distinctive species described recently from China, *A. coronarius*. Many specimens differing hardly at all in morphology and only slightly in coloration, were represented in the TIGER Malaise trap samples, but barcoding revealed this to be a complex of 15 molecularly well-separated species, but we were unable to determine whether any were the same as the Chinese species and so we took the risk of describing all of them. Further, along with a few other very morphologically uniform sets, some parts of the key rely on molecular characters, either wholly or in part, and for the *A. coronarius* group alternative key routes were given for specimens that either had barcodes or did not, in the latter case, only a subset of the species could be recognized morphologically.

Was turbo-taxonomy quicker? Indeed for the descriptive part it was a major boon – imaging (typically 5 or 6 views) took about 30 minutes per species, with approximately a further 20-30 minutes of 'photoshop ®' tidying up, the same for arranging plates, and approximately the same for noting the character states that would be presented as text. Additional work was involved in mounting specimens and recording collection data, again about 30 minutes. So all in all, preparation and description took approximately 2 hours per species.

What the above hides, however, was the very considerable amount of time, greatly more than all the descriptions combined, writing, checking, re-writing, etc., the identification key. We do not think that there is any substitute for experience and expertise in this aspect.

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Top 10 DNA Barcoding Publications 2013

Measured using Publish or Perish (Jan-Oct)

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

1. Santoferrara LF, McManus GB, Alder VA (2013) Utility of genetic markers and morphology for species discrimination within the order Tintinnida (Ciliophora, Spirotrichea). *Protist* 164: 24-36.
2. Souffreau C, Vanormelingen P, Van de Vijver B, Isheva T, Verleyen E, Sabbe K, Vyverman W (2013) Molecular evidence for distinct antarctic lineages in the cosmopolitan terrestrial diatoms *Pinnularia borealis* and *Hantzschia amphioxys*. *Protist* 164: 101-115.
3. Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. *PLoS ONE* 8: e66213.
4. Baselga A, Fujisawa T, Crampton-Platt A, Bergsten J, Foster PG, Monaghan MT, Vogler AP (2013) Whole-community DNA barcoding reveals a spatio-temporal continuum of biodiversity at species and genetic levels. *Nature Communications* 4:1892.
5. Walther G, Pawlowska J, Alastruey-Izquierdo A, Wrzosek M, Rodriguez-Tudela JL, Dolatabadi S, Chakrabarti A, de Hoog GS (2013) DNA barcoding in Mucorales: an inventory of biodiversity. *Persoonia-Molecular Phylogeny and Evolution of Fungi* 30: 11-47.
6. Staudacher K, Schallhart N, Pitterl P, Wallinger C, Brunner N, Landl M, Kromp B, Glauning J, Traugott M (2013) Occurrence of *Agriotes* wireworms in Austrian agricultural land. *Journal of Pest Science* 86: 33-39.
7. García-Robledo C, Erickson DL, Staines CL, Erwin TL, Kress WJ (2013) Tropical plant-herbivore networks: reconstructing species interactions using DNA barcodes. *PLoS ONE* 8: e52967.
8. Decaëns T, Porco D, Rougerie R, Brown GG, James, SW (2013) Potential of DNA barcoding for earthworm research in taxonomy and ecology. *Applied Soil Ecology* 65: 35-42.
9. Stensvold CR (2013) Comparison of sequencing (barcode region) and sequence-tagged-site PCR for *Blastocystis* subtyping. *Journal of Clinical Microbiology* 51: 190-194.
10. Shen Y-Y, Chen X, Murphy RW (2013) Assessing DNA Barcoding as a tool for species identification and data quality control. *PLoS ONE* 8: e57125.