


international
BARCODE
OF LIFE



Barcode BULLETIN

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Spiders of New Zealand: Barcoding a Species Diverse and Largely Endemic Group

Applications

Assessing
Pollen Diversity
Through Next-
Gen Sequencing

Research

Data Release
Promotes
Collaboration
and Discovery

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

Another year has passed and we are looking ahead to 2015 which marks the end of iBOL's first five years. The Barcode Bulletin accompanied the project all along and we can proudly say that our little newsletter captured a lot of the diversity of ideas, projects and events that made iBOL a success.

The coming year will also be the year of the 6th International Barcode of Life conference and within this issue you'll find more details about this event. In addition we share the newest on research and application from six continents. Once more, a truly global issue of the Barcode Bulletin that is both entertaining and informative.

What would be a new year without any new year's resolutions? As for the team of the Barcode Bulletin, we will strive for the continuation of our high level of quality but also to increase our impact within and beyond the research community. DNA barcoding deserves more attention than it currently receives and we like to do our part to change this.

We wish you Happy Holidays and a healthy and prosperous New Year.

Dirk Steinke
Editor-in-chief

News Briefs

The Secretariat of the Convention on Biological Diversity will be launching a capacity-building "training for trainers" course in DNA barcoding that will be made available to interested Parties to the Convention, thanks to financial support from the Government of Japan. This activity will be facilitated by the Biodiversity Institute of Ontario and will focus on building technological capacity to help Parties achieve Aichi Biodiversity Target 9, specifically, detection and monitoring of invasive alien species (IAS).

This course will include a distance education module followed by hands-on training at the CCDB for selected national experts. It will cover standard operational workflows used in barcoding of IAS and will provide participants with specialized skills that can be used to apply this technology and approaches in their home countries. Training is geared toward professionals involved in national regulatory and monitoring organizations overseeing quarantine and management of IAS. The course is also open to self-funded experts from countries that are not eligible for SCBD financial support.

It is expected that the call for applications will be announced in January 2015 on the CBD website (<http://www.cbd.int>) and training will commence in March of 2015. For inquiries on eligibility, selection criteria and application procedure, please contact the CBD Secretariat at secretariat@cbd.int.

Registration is now open for the next offering of the online course "Introduction to DNA Barcoding", developed by Barcode Bulletin editor Dirk Steinke in conjunction with the Centre of Open Learning at the University of Guelph. The eight week course begins on March 2, 2015 and covers a range of topics including barcoding workflows and applications. For more information visit www.dnabarcodingcourses.com. Space is limited to ensure a comprehensive and valuable experience for all participants.

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Paradictyna sp.
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Barcoding New Zealand Spiders

Written by: Ian Hogg (University of Waikato)

With two species of native land mammal – both bats – and only 131 species of land-based birds, New Zealand is often considered to be a species-poor land mass. However, with an estimated 2000 species of spiders, invertebrate taxa are the exception to this rule. Such species richness compares favourably with much larger land masses such as that of North America, which has around 3500 species.

A collaborative project has recently begun in New Zealand to catalogue and barcode both the endemic and introduced spiders. The initial goal – now completed as part of the *A Photographic Guide to Spiders of New Zealand* – was to barcode the 90 species of spiders most commonly encountered in New Zealand. This ongoing project involving researchers from the University of Waikato,

Canterbury Museum and the Biodiversity Institute of Ontario, now aims to barcode a further 1200 specimens from throughout New Zealand before March 2015. Two summer research students, Nigel Binks and Annie West at the University of Waikato are working in conjunction with naturalist and photographer Bryce McQuillan and are undertaking the bulk of the collecting/curating work.

“Despite the estimated 2000 species of spiders, only 1134 are currently described and many specimens can only be assigned to family level.”

Spider systematist Cor Vink at the Canterbury Museum is also undertaking collecting in New Zealand’s South Island and providing his expertise on the identification of specimens. All data are being added to BOLD and will contribute to the New Zealand Barcode of Life (NZBOL) programme and ultimately enable comparison with extensive datasets such as those available on BOLD.

From the specimens collected thus far we have found high intraspecific morphological variability based on age, sex and likely environmental factors. For example, the *Badumna* genus, commonly known as the grey house spider, shows a wide range of colour morphs among specimens. Once considered a single species, we now know that the grey house spider is characterized by at least three separate species, including two described species (*Badumna longinqua*, *Badumna insignis*) and one currently undescribed species. Such variety renders morphological identification somewhat challenging particularly for novice collectors.

“...we have found high intraspecific morphological variability based on age, sex and likely environmental factors.”

Despite the estimated 2000 species of spiders, only 1134 are currently described and many specimens can only be assigned to family level. With 95% of species thought to be endemic, accurate identification of New Zealand's spiders will require the robust coverage of taxa envisaged as part of this research project.



Arachnura feredayi
© Bryce McQuillan



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Image credit: Olga Schmidt

ISCC 2014 Meeting: Planning the Future of iBOL

Written by: Axel Hausmann (Bavarian State Collection of Zoology)

iBOL's International Scientific Collaboration Committee (ISCC) held its annual meeting from October 24-26, 2014 at the Bavarian State Collection of Zoology (ZSM) in Munich, Germany. A group of 26 delegates and associates from 18 iBOL nations discussed achievements over the past four years as well as iBOL's future.

Based upon a brief summary of progress by Paul Hebert, the ISCC members concluded that iBOL will achieve its primary goal of delivering barcode records for 500,000 species before 2016, assuming that there will be a dedicated effort to move currently private records into BOLD and/or GenBank. It was also concluded that it might be possible to achieve this milestone without analyzing 5 million specimens.

Stimulating subsequent discussions revolved around three major challenges iBOL faced during its first five years: 1) obtaining and processing

millions of voucher specimens, 2) generating barcode sequences from them, and 3) storing, managing, and analyzing sequences and associated data in a web-accessible, scalable database system. Reports from all 18 nations indicated that both data generation and barcode application

are growing substantially, particularly in Germany and Canada. The director of the ZSM, Gerhard Haszprunar, provided a detailed overview of the barcode efforts of the ZSM and their political impacts in Germany. Axel Hausmann summarized the results of six years barcoding in two major projects, the fauna of Bavaria and the fauna of Germany. Stefan Schmidt described techniques of associated integrative taxonomy and introduced plans for major barcoding projects in Indonesia.

"Reports from all 18 nations indicated that both data generation and barcode application are growing substantially..."

Sujeewan Ratnasingham introduced the delegates to exciting new informatics developments. He showcased features that will appear in the next version of BOLD, including a new platform to support the analysis of Next-Generation Sequencing (NGS) data. In addition, participants discussed the LifeScanner project and its potential to accelerate the development of a mobile barcode device. The progress and applications of NGS technologies were discussed by Mehrdad Hajibabaei and there was general support for reinforced efforts to employ this technology for large-scale bio-surveillance programs.



required funds. Given these positive impacts, the delegates agreed to work towards extending iBOL for another five years. It was concluded that efforts in library construction should continue, but that Phase II would need to focus on the application of DNA barcoding to address important societal issues. It was agreed that the goals of iBOL phase II should be outlined at the 6th International Barcode of Life Conference in August 2015.

“...the delegates agreed to work towards extending iBOL for another five years.”

The ISCC also indicated its support for the establishment of an International Society for DNA Barcoding as announced in Kunming in November 2013.

Members of the ISCC agreed that iBOL's structure had made it possible to achieve a major scientific milestone and to gain the It was suggested that the International Union of Biological Sciences might provide an umbrella organization for the new society.

Blue Planet Prize Presented to Daniel Janzen

In recognition of his remarkable achievements in biodiversity research and conservation, Prof. Daniel Janzen was awarded the Blue Planet Prize for 2014. The international prize, which is sponsored by the Asahi Glass Foundation and includes a commemorative trophy and a ¥50 million award, was jointly presented to Prof. Janzen and the Instituto Nacional de Biodiversidad of Costa Rica (INBio) on November 12th in Tokyo.

Through this award, Prof. Janzen will continue to shape this world by promoting sustainability, restoration, and, above all, bioliteracy because, in his words, “without bioliteracy, nature is just a green threatening mass and there is little hope of its peaceful coexistence with all of us”.



From left to right: Chairman Tetsuji Tanaka, Prof. Daniel Janzen, and Dr. Rodrigo Gamez Lobo
(President of the Instituto Nacional de Biodiversidad).



Image credit: flickr.com/photos/cisphotos/

The Sixth International Barcode of Life Conference

The 2015 International Barcode of Life Conference exemplifies the homecoming of DNA barcoding. Taking place from August 18 to 22, 2015, the conference will celebrate the many achievements of the first phase of the project and will usher in the next chapter of DNA barcoding research. Boasting state-of-the-art conference facilities, beautiful architecture, and friendly green spaces, the conference will be hosted by the University of Guelph, located in Guelph, Ontario, Canada. This venue is conveniently situated near several world-renowned attractions, such as the CN tower, Niagara Falls, and the Royal Ontario Museum.

A wide selection of workshops and tours will precede the conference on Monday, August 17 and four full days of plenary, parallel and

poster sessions as well as evening events will follow. Evening events include a Gala dinner in neighbouring Cambridge and a public session in scenic downtown Guelph that will focus on the status of biodiversity at national and global levels through presentations and a panel discussion. A keynote address by Daniel Janzen, winner of the Blue Planet Prize for 2014, will bring the conference to a close on August 21, with post-conference excursions planned for Saturday, August 22.

Conference registration will open in January with an abstract submission deadline of April 15 for both oral and poster presentations. More information on registration, abstract submission, workshops, plenary speakers, and special symposia is available at dnabarcodes2015.org.



Image credit: Craig Bain



Image credit: Richard Bain



Image credit: Richard Bain

Building Peru's National DNA Barcoding Expertise

Written by: Alex Borisenko, Muhammad Ashfaq, Emily Berzitis and Dirk Steinke (Biodiversity Institute of Ontario)

The project “DNA Barcoding to support Biodiversity Conservation, Sustainable Harvesting and Trade in Peru” has reached another milestone with the completion of advanced training of Peruvian experts in Canada. This project is jointly executed by the Peruvian Museum of Natural History (MHN-UNMSM) and the Biodiversity Institute of Ontario, with funds provided by Canada's DFATD and the Conference Board of Canada under the CATRTA Program (for more details, see the [Barcode Bulletin March Issue](#)).

Ten participants from Peru's first National Workshop in DNA Barcoding were selected to take the online training course “[Introduction to DNA Barcoding](#)” through the University of Guelph's Centre of Open Learning in April-May 2014. Six of these experts visited the Biodiversity Institute of Ontario in Guelph in June-August 2014 to solidify the acquired theoretical knowledge with practical hands-on experience in standard DNA barcoding protocols and workflows. The training participants represented a diverse cross-section of the country's research and regulatory establishments: from academic research institutions (San Marcos University) and NGO's (CORBIDI) to regulatory agencies (SENASA, ITP), reflecting the diversity of barcode approaches and applications.

Training took place in BIO's dedicated training facility at the Canadian Centre for DNA Barcoding and covered all aspects of the standard DNA barcoding workflow from collection management through laboratory work and data analysis; it also included an in-depth tour of the CCDB's high throughput core analytical facility. Training modules were delivered by BIO's research staff, taking into account the research interests and institutional profiles of the participants, as well as relevant priority areas of application. In addition, visitors had the opportunity to meet with BIO's faculty, research and technical staff and discuss both scientific and practical aspects of DNA barcoding. An important outcome of this exercise is the population of the reference DNA barcode library for Peruvian species of key conservation, regulatory and economic importance.

Following their return to Peru, participants held several meetings with the Peruvian project leaders to outline their involvement in coordination and advancement of future DNA barcoding activities in this country; they also gave interviews to local media. The project has now entered its final phase which will see the development of the national DNA barcode strategy for Peru.

Pollen DNA Barcoding Using Next-Generation Sequencing

Written by: Alexander Keller, Stephan Härtel, and Ingolf Steffan-Dewenter (University of Würzburg)

Identification of pollen plays an important role in ecology, particularly in studies about mechanisms of plant–pollinator interactions, resource use of flower-visiting animals and the identification of historical plant communities. The morphology of pollen grains often displays species-specific characteristics, with diverse structures and sculpture, which can be used by experts to identify taxa. Yet, for many taxa it remains still difficult to delineate between closely related species when using light microscopy. Consequently they are pragmatically grouped at higher taxonomic levels. This strongly limits data analyses on pollen diversity.

DNA barcoding has often and successfully been applied to all major groups of organisms, including plants. The application of DNA-based methods for pollen identification has also substantially increased and shows great potential especially with difficult to delineate and fossil taxa. This becomes difficult when one tries to directly determine the diversity of organisms in environmental samples which comprise mixtures of several species. Even for DNA barcoding, such samples need to be

manually separated using optical methods as well as individually amplified and sequenced. This procedure is still error-prone and tedious, and thus not very efficient.

Assessments of pollen diversity would therefore benefit from a more effective method of species-level determination from mixed samples. In particular the ability to address larger counts, higher processing speed, improved objectivity and automation would be highly attractive for large-scale applications.

“...for many taxa it remains still difficult to delineate between closely related species when using light microscopy.”

In our recent study published in *Plant Biology* we thus evaluated performance and reliability of amplicon-based next-generation sequencing and automatic bioinformatical classification for this task by directly comparing sequencing-based data with data obtained from light microscopy for pollen collections of the honey bee (*Apis mellifera*) and a solitary bee species (*Osmia bicornis*).

With this novel approach, we were able to considerably improve pollen diversity assessments in comparison with traditional optical microscopy, i.e. novel taxa were identified and general classification-deepness of taxa was improved. Thus, our approach expanded the possibilities of distinguishing between closely related species within a genus. Also, molecular assessments were successful for solitary bee nest samples, where samples included pollens as well as contaminating material (feces and soil).

“...next-generation sequencing has great promise as an alternative method in palynology...”

A general limitation of DNA barcoding however, are missing taxa or inadequate sequence representation in the database used.

We conclude that next-generation sequencing has great promise as an alternative method in palynology with various potential applications arising from basic ecology and applied environmental and agricultural research. These comprise studies of pollen material of various origins, including plants, pollinators, soil and air-born samples, but also quality control of bee products such as honey, pollen, propolis and food produced therefrom. The results of such assessments are also of great importance for identifying the diversity and specialization of plant–pollinator interaction networks, the worldwide origin of samples and ecosystem services.

Furthermore, the methodology may be equivalently applied to other questions not only related to pollen, as e.g. naturally occurring communities of algae, food or mixed probes of plant tissue fragments. It is a useful technique, broadening pollen assessment capabilities from expert labs to all work groups with access to standard molecular laboratory equipment.

The used training set, alongside installation and application notes, is available for download at www.dna-analytics.biozentrum.uni-wuerzburg.de.

For more information about the results discussed in this article, see DOI: [10.1111/plb.12251](https://doi.org/10.1111/plb.12251)



Image credit: Susanne Schiele
(University of Würzburg)



Image credit: Susanne Schiele
(University of Würzburg)

Data Release Furthers the Reach of DNA Barcoding

Written by: Chandra Moffat (University of New Brunswick)

A key component of the global DNA barcoding initiative is a community-wide commitment to the rapid release of DNA barcode data prior to publication (following the Fort Lauderdale Principles – Wellcome Trust 2011). In order to achieve this goal, “data producers” must agree to make barcode data immediately and freely available, and “data consumers” must acknowledge the source of the data being used, despite it not having been published.

Findings provide a case for the public release of DNA barcode data prior to taxonomic annotation.

Our recent study highlights the benefits of releasing not only pre-publication DNA barcode data in public databases, but DNA barcode data even prior to taxonomic annotation, the release of which is controversial.

This story begins a few summers ago when, in the course of my MSc degree (a collaborative project between UBC Okanagan, CABI Europe-Switzerland www.cabi.org and Agriculture and Agri-Food Canada), I travelled to Central Europe in search of potential biological control agents for a group of plants invasive in North America,

Pilosella hawkweeds. This genus includes common species such as orange hawkweed (*P. aurantiaca*) and meadow hawkweed (*P. caespitosa*), which invade highly disturbed areas (e.g. meadows, roadsides, and deforested areas).

The economic impact of these invasions is estimated in the tens of millions of dollars, and a non-chemical control method is sought for ecological and economic reasons. A biological control program to find and release specialized natural enemies of hawkweeds was initiated in their native Europe.

A promising candidate biological control agent for hawkweeds is the gall wasp *Aulacidea pilosellae* Kieffer (Hymenoptera: Cynipidae) which induces small galls on hawkweeds, diverting plant nutrients away from growth and reproduction. Part of my investigation involved determining if there was any divergence among wasps collected from different host plant species, or in different parts of the geographic range, as previous ecological data suggested this may be the case.

I collected galled plants and sequenced the *A. pilosellae* larvae at both the COI region and the nuclear gene region 28S-D2. In a routine comparison of these DNA sequences in GenBank (BLASTn), I expected the most similar sequences to be from other species of *Aulacidea* already in GenBank. Instead, the most similar sequence returned was from a hymenopteran specimen

identified only to order and collected in eastern Ontario, Canada.

“Such a release enabled collaboration, and most importantly, the documentation of a species new to North America.”

Surprised by the BLASTn result, I contacted the collector of the specimen, Alex Smith at the University of Guelph. Alex confirmed the collection location, and sent me a photograph of the specimen, which indeed looked strikingly like my *A. pilosellae* from Europe. Through email, we arranged for Alex to sequence his specimen (GenBank JN28873, BOLD:AAU8720) at the same nuclear gene region as I had sequenced my *A. pilosellae* (28S-D2). The second gene region confirmed the COI results: the specimen collected in eastern Ontario shared an identical 28S-D2 sequence to my European *A. pilosellae*, making ours the first record of the species in North America.

Detecting *A. pilosellae* may have relevance for the hawkweed biological control program – the approval process for this candidate agent may

be influenced as any biological control program related releases would not mean introducing the species into North America for the first time.

The detection of this species in North America was only possible due to the release of the DNA barcode data from the Ontario specimen prior to taxonomic annotation. While such releases are controversial, in our case, such a release enabled collaboration, and most importantly, the documentation of a species new to North America. We hope our story encourages more data producers to release their barcode data prior to taxonomic annotation, and for the appropriate databases that store such data to accept data prior to taxonomic annotation.

For more information about the results discussed in this article, see DOI: [10.4039/tce.2014.48](https://doi.org/10.4039/tce.2014.48)



Above: *Aulacidea pilosellae* collected in a Malaise trap in Wilberforce township, Ontario. Below: Collecting galled *Pilosella caespitosa* in a roadside ditch in Germany



Successful Use of DNA Barcoding in International Trade Dispute

Written by: Qiaoyun YUE and Jian Chen (Zhongshan Entry-Exit Inspection and Quarantine Bureau Technology Center, Guangdong, China)

Tea bags made from barley leaf powder are a very popular healthy organic food commodity in Japan and Korea. They are rich in dietary fiber, starch, protein, fat, vitamins B1 and B2, calcium, phosphorus, iron, phenolic compounds and other nutrients. The *Hordeum vulgare* processed tea is believed to stimulate the digestion of greasy food and to provide additional gastrointestinal benefits with continuous use. *H. vulgare* is one of the oldest species of Chinese grain, having first been planted in China approximately 5,000 years ago.

At present, China is the world's main producer of *H. vulgare* and exports large amounts of barley tea every year. In recent years, food fraud, including barley tea adulteration, has become both more sophisticated and frequent which seriously harms the interests of consumers and negatively impacts foreign trade.

In our work, DNA barcoding was adopted to investigate barley tea products that were exported to country X from China by a Chinese company and returned due to suspected adulteration with other plant components. Identification of the species based on morphology was impossible because the barley leaf had been crushed into ultra-fine powder ($D < 38\mu\text{m}$ of the crushed grain).

We amplified the four commonly used DNA barcode fragments for plants (*rbcL*, *matK*, *trnH-psbA* and ITS2) to determine the composition of the returned barley tea, thereby providing data for the resolution of the trade dispute between the Chinese company and country X.

"...barley tea adulteration has become both more sophisticated and more frequent..."

We found that 10 of 13 (76.9%) batches were contaminated with other plant material including *Morus* sp. (Mulberry), *Triticum* spp. (wheat), *Avena* sp. (oats) and *Chenopodium ficifolium* (goosefoot).

Goosefoot and wild oats are common nuisance weeds and often appear in barley fields. Therefore they may unintentionally be included

While contamination by oats, goosefoot, and wheat may be unintentional, addition of mulberry is likely deliberate.

during the barley leaf harvest process. Due to the similar seed and plant morphology of wheat and barley, barley seeds might be contaminated with wheat, leading to the detection of the latter.

Mulberry, however, is quite different from the barley in morphology and deliberate adulteration may have occurred in an attempt to reduce costs and achieve maximum profit.

We also developed an alternative protocol for amplification of *matK*, which is present in the cell as single copy and is difficult to amplify with the common protocols. *matK* was easily amplified in all of our 13 batches using our new protocol. For details, please see DOI: [10.2478/dna-2014-0004](https://doi.org/10.2478/dna-2014-0004).



Image credit: Jian Chen

Barley tea powder.

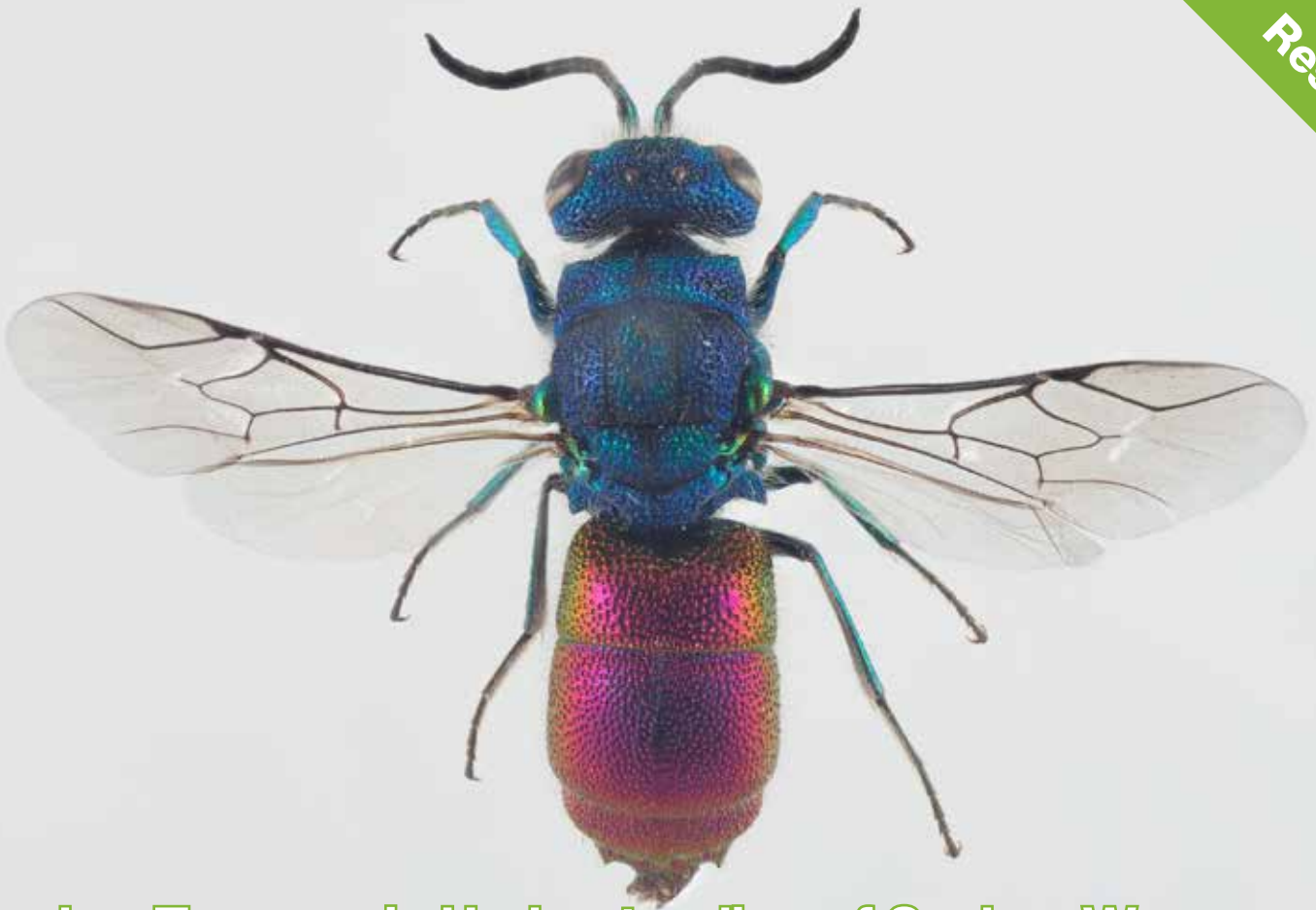


Image credit: flickr.com/photos/simonpu/

Above: *Triticum* spp. (Wheat) Below: *Morus* sp. (Mulberry)



Image credit: flickr.com/photos/31035796522@N01/



Chrysis ignita
Image credit: A. Staverlökk/NINA

Towards a Taxonomic Understanding of Cuckoo Wasps

Written by: Frode Ødegaard (Norwegian Institute for Nature Research), Juho Paukkunen (Finnish Museum of Natural History), and Villu Soon (University of Tartu)

“For almost 200 years, taxonomists have struggled with sorting out biological species in this group.”

The cuckoo wasps or gold wasps, with their bright and shiny colors, are among the most beautiful insects on Earth. Despite being attractive to collectors and nature enthusiasts, the taxonomy of Chrysididae remains poorly understood. In many cases, variation within species is higher than between species and diagnostic characters have been difficult to find. For almost 200 years, taxonomists have struggled sorting out biological species in this group.

Worldwide there are almost 3000 described species of cuckoo wasps. They are all cleptoparasites and recent studies showed that there is a high degree of host specialization among species. Hosts in our study region (northern Europe) consist of solitary wasps

and bees (mostly Eumeninae, Crabronidae and Megachilidae) while members of the subfamily Cleptinae parasitize on sawflies.

The largest genus, *Chrysis*, with an almost worldwide distribution, includes several unresolved taxonomic complexes. In northern Europe, the *Chrysis ignita* group has received particular attention as extremely problematic. Already in 1836, M.A. Shuckard made an early attempt to split *Chrysis ignita* into separate taxa.

Intensive work by several taxonomists of the 19th and 20th centuries sorted out some more or less obvious taxa but many taxonomic decisions mainly relied on personal opinions of the authors rather than general consensus.

“...the use of DNA markers has proven essential for delimiting closely related hymenopteran parasitoids.”

With recent progress in molecular biology, the use of DNA markers has proven essential for delimiting closely related hymenopteran parasitoids. Particularly, the COI sequence of mtDNA has shown to be useful for resolving cryptic taxa in combination with morphological and ecological evidence. An analysis of the barcodes of 364 specimens belonging to the *Chrysis ignita* group, representing all the 18 taxa known from northern Europe, gave high support for 15 species (Soon et al. 2014, DOI: [10.11646/zootaxa.3786.3.4](https://doi.org/10.11646/zootaxa.3786.3.4)). However, two species were not resolved, another two species might include additional cryptic species, and finally, three separate clusters might represent additional undescribed species.

“...DNA barcoding appears highly suitable for assisting both species delimitation and identification in this group.”

National initiatives of DNA barcoding in Norway (NORBOL) and Finland (FINBOL) include a strong focus on aculeate hymenopterans and, with additional contribution from

research projects in the Baltic countries, most species occurring in the region are currently represented by COI sequences in BOLD. A comprehensive study of the Chrysididae occurring in Fennoscandia, Denmark and the Baltic countries, including extensive barcoding of all taxa, made a significant contribution to the recent review of the species in the region (Paukkunen et al. 2014, DOI: [10.11646/zootaxa.3864.1.1](https://doi.org/10.11646/zootaxa.3864.1.1)). A total of 73 species of Chrysididae are listed and a new species for the region, *Elampus foveatus*, was revealed by DNA barcoding.

Results from the same study give indications that additional cryptic species are hidden among other taxa, for instance, in the genus *Omalus*. The taxon, *Omalus puncticollis*, has been discussed for a long time and several authors believe that it should be synonymized with *O. aeneus*. However, our barcoding results indicate that it is a good species well separated from other clades.

On the other hand, specimens identified as *Omalus aeneus* s.str. group into five clearly separated clades in our region. We are currently trying to sort out if these clades represent separate species by looking for conclusive morphological evidence.

Considering the difficulties that often arise during species determination of Chrysididae based on morphological characters, DNA barcoding appears highly suitable for assisting both species delimitation and identification in this group.





Let's Share Our Science

Written by and images by: Morgan D. Jackson @BioInFocus (University of Guelph)

Politicians proudly proclaim, "I'm not a scientist". Federal scientists are barred from discussing results by layer upon layer of bureaucratic obfuscation. Scientific "documentaries" on TV contain more fiction than fact.

In the battle between scientific discovery and anti-science sentiment, it's sometimes difficult

The 3 B's of Social Media #SciComm:

Be Yourself
Be Honest
Be Engaged

to tell who's coming out ahead. But what can we, the practicing scientists making the discoveries, do to help change the narrative, and with it, public perception?

For starters, we could engage with the public about our science *directly*, using any one of the dozens of open-access publication services that have sprung up in the past decade, and I don't mean *PLoS ONE* or the *PeerJ*.

No, I'm speaking about **social media**.

With an engaged, worldwide user-base of billions of people, social networks like Facebook, Twitter, YouTube and Instagram provide outlets for sharing your work directly with an audience already primed to Like, Retweet, and Share science with their family, friends, and followers.

Never before have scientists had this kind of opportunity to engage with a global audience hungry for updates from the brightest minds, reports from the cutting edge, or even glimpses into what it's like to be a scientist in the 21st century.

"...it won't be long until we can reap the benefits of an informed citizenry that values science..."

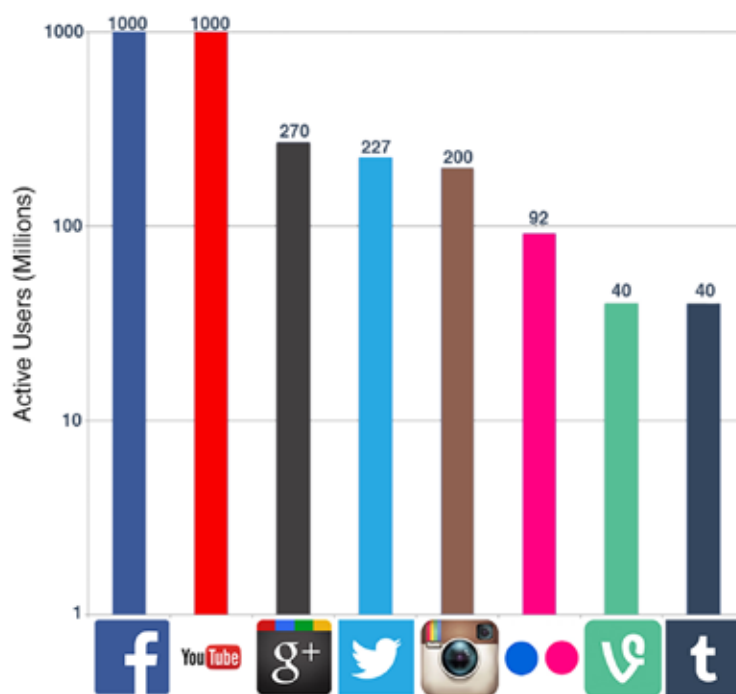
While Bik & Goldstein (DOI: [10.1371/journal.pbio.1001535](https://doi.org/10.1371/journal.pbio.1001535)) provide an excellent overview of how social media can be used by scientists, each network has its own quirks, and the best way to learn is to dive in and start experimenting to see which one best matches your goals. If you're trying to engage with your local community and stakeholders, Facebook will allow you to piggyback your message onto your real-world social networks. If you only have short breaks throughout the day to share snippets of your science, Twitter's 140-character limit can allow you to make a meaningful contribution without investing hours in wordsmithing. In the biodiversity sciences, fieldwork and specimens can make compelling material for any one of the image-based social networks like Instagram, Vine, or YouTube.

If nothing else, why not write plain-language summaries (Buddle, [2012](#) & [2013](#)) of your publications, including the story of how your research fits into the larger picture, and why it's important to society as a whole? Many professional societies, like the Entomological Society of Canada, maintain blogs that are fueled by submitted contributions such as these. You can also ask individual bloggers in your subfield if they accept guest posts, or better yet, start your own blog and stake out your own little corner of the internet. In years past, scientists sent, by snail- or e-mail, reprints of their work to colleagues; plain-language blogging simply represents the natural evolution of this process in a globally-connected world.

Of course, science communication shouldn't be limited to digital media: don't underestimate the power of human connection. Introduce your community to the natural history of your local park by organizing a BioBlitz, and use the opportunity to explain why biodiversity research and biological collections are important. Share the impact and implications of mislabeled sushi with your fellow commuters on the subway. Give the gift of science this holiday season: family meals provide a captive audience too polite, or too hungry, to walk away while you

explain that great new research paper that was recently published!

When it comes to science communication, the only thing standing in your way is your imagination. While academia may be slow to acknowledge the value and time commitment of scientists sharing their work publicly, it won't be long until we reap the benefits of an informed citizenry that values science because of it. And with luck, we'll inspire more people to proudly proclaim, "I *am* a scientist".



Above: Current active users of major social networks, as reported by each company.

Below: Chris Earley of the University of Guelph Arboretum sorts BioBlitz samples while engaging with an enthusiastic crowd.



DNA Barcoding: the Indian Scenario

Written by: Swapnil Gaikwad (Savitribai Phule Pune University), Sanket Tembe, Yogesh Shouche (National Centre for Cell Science) and Hemant Ghatge (Modern College)

India is one of the most biodiversity rich countries, home to hotspots like the Western Ghats and the Himalayas. In spite of this rich biodiversity heritage, well documented in the Fauna of British India volumes and having many endemics in all groups, much still remains to be understood about it. Many species are difficult to identify and are poorly known.

We believe that a rich DNA barcode database, containing well identified species, would help species identification. Successful and sustained collaboration between taxonomists and molecular biologists is essential to achieve this goal.

“There has been a dramatic increase in DNA barcoding activity in India...”

The first initiative in DNA barcoding was led by the Department of Biotechnology (DBT), India, to barcode species of butterflies and amphibians from the Western Ghats of India. Barcoding was conducted by the National Centre for Cell

Science (NCCS), Pune and Modern college, Pune, as well as the Systematics Lab at the University of Delhi. A DNA barcode library of around 120 species of butterflies from Western Ghats was generated by this project. In addition, through this initiative, 14 new species of dancing frogs were described.

There has been a dramatic increase in DNA barcoding activity in India and more and more projects are being launched. Major momentum for DNA barcoding in India came with the announcement of funding of 10 million dollars by the Director General of the Indian Council for Agricultural Research (ICAR) for barcoding work on plants, insects and fishes of agricultural importance.

DBT also provided funding to open the ‘Paul Hebert Centre’ for DNA barcoding and biodiversity studies at Babasaheb Ambedkar Marathwada University, Aurangabad, for training researchers and conducting barcoding research.

With the availability of such resources, many projects were initiated by researchers all over the country. Through these initiatives, DNA barcoding helped to identify new species of grass from Tamilnadu and also helped to identify epi-endophytic green algae *Ulvella leptochaete* (Ulvellaceae, Chlorophyta) for the first time from India. Additionally, DNA barcoding has been applied successfully for the identification of: herbal juices of medical importance, edible mushrooms consumed by the tribes of Meghalaya, wood of threatened and commercial tree species, and mosquitoes as well as sandfly species.

The Department of Biotechnology, Assam University, has undertaken a major initiative to barcode fishes. This group has barcoded 170+ species of fishes for the first time and also designed a mini barcode primer for the assessment of archival biodiversity of fishes. Furthermore, they showed that a species of *Neolissochilus* is distinct from *Tor progeneius* / *T. putitora*. Significant work is underway on freshwater fishes but marine fishes must also be investigated.

In collaboration with the Indian Navy and Air Force, NCCS is now utilizing DNA barcoding to identify birds killed by airplanes. The remains of a bird after such a strike are generally limited to a smear of blood, feathers or fragments of tissues and, thus, morphological identification is impossible. They have received more than 100 birdstrike samples in the form of a blood spot or damaged tissues and, out of these samples, they could identify 70 species with DNA barcoding, using sequences available on BOLD for comparison.

Although the overall scenario in India is encouraging, it is essential that we look at problematic invertebrate groups such as termites, buprestids and cerambycids that damage precious wood. These groups are also difficult to identify for various reasons. Similarly,

Future efforts should focus on problematic invertebrate groups, commercial seafood organisms, and medically useful plants.

efforts must be directed at barcoding commercial seafood organisms (algae included) so that one can verify the purity of canned material. All medically useful

plants must also get priority as herbal medicines are widely used and duplicates are widespread.

We also expect the establishment of a common web portal to host all information with respect to DNA Barcoding of Indian Faunal / Floral elements as well as the development of facilities for the national repository of voucher specimens to avoid confusion created by misidentified species.

Cerambycidae need urgent attention in India.



Image credit: Hemant Ghate

Facilities for careful storage and maintainance of vouchers is of utmost importance.



Image credit: Hemant Ghate



Photo courtesy of HudsonAlpha

Student Program Provides First Barcode of a Rare Plant

Written by: Jessica Hunter (Hazel Green High School, Alabama, USA) and Madison Stone (Madison County High School, Alabama, USA)

Over the summer of 2014 we were given the opportunity to attend HudsonAlpha's Biotech Academy located in Huntsville, Alabama. It was a prestigious opportunity because only one student was chosen from each of the 14 area high schools. The Biotech Academy was a four-week intensive program that gave us the chance to work alongside professional scientists and educators in a hands-on environment where we learned about the foundations of molecular genetics, DNA barcoding, genetic engineering, and synthetic biology.

One of our labs was to isolate DNA from plant tissue. Our group was assigned *Clematis morefieldii*, also known as Morefield's Leatherflower. This plant is rare and on the U.S. Endangered Species List. It can only be found in North Alabama and Southeast Tennessee.

"It can now be viewed worldwide and compared to other species."

With the help of Harvey Cotton, the chief horticulturist at Huntsville Botanical Gardens, we collected our sample of several leaves off this plant's vine. After extracting DNA from our plant sample, we used PCR to amplify a portion of the DNA. After we analyzed our sequence we realized it had not yet been published in any DNA database so we submitted the sequence to GenBank. It can now be viewed worldwide and compared to other species.

Clematis morefieldii is a vine-like plant that can grow up to sixteen feet long. The most distinguishing feature of the plant is the bell shaped purple-pink flower grown on its vine during the spring and summer months. In order for Morefield's Leatherflower to properly grow and produce it must be in the presence of limestone in a wooded area.

Due to this plant being on the endangered species list, a five-year study was published by the U.S. Fish and Wildlife Services in the 1990s. During this study the plant's environmental effects were recorded. This plant is used as one of several food

A recovery plan for this endangered species included a protected population at Huntsville Botanical Gardens.

sources for small animals, birds, and insects that live in the same area of this plant.

After the five-year study was completed, a recovery plan was established to preserve

Morefield's Leatherflower. This plan requires for ten populations of this plant to be protected from all foreseeable threats in order to allow the plant to grow and reproduce as it would naturally. One of the recovery locations for Morefield's Leatherflower is Huntsville Botanical Gardens. Due to this plan being in place we were able to have easy access to Morefield's Leatherflower to gain a sample for sequencing.

We are very grateful to HudsonAlpha for giving us an amazing opportunity to have a lasting impact on the world of science by

sequencing Morefield's Leatherflower. We believe it is very important to have DNA samples of all living things, especially endangered species. We are thankful to have been a part of documenting and researching such a rare plant.

View the *rbcL* sequence (GenBank KM593689.1) at: <http://www.ncbi.nlm.nih.gov/nuccore/KM593689>



Photo courtesy of HudsonAlpha



Photo courtesy of HudsonAlpha

Optimizing Collection Methods for Successful Barcoding

Written by: Sandi Willows-Munro (University of KwaZulu-Natal)

The proper collection and storage of biological material in a "DNA friendly" way is important for barcoding and other molecular studies. Factors such as the age of the specimen, how specimens are stored and DNA extraction protocols all play a role in the success of DNA barcoding. How specimens are collected can also play a role in the success of molecular applications but exactly what effect the killing method has on sequence quality is not well known.

Direct immersion and storage in alcohols such as ethanol and isopropanol is the most popular method of killing and storage in insects. This method is not useful in taxa such as Lepidoptera where fragile morphological features such as scales on the wings can be damaged by wet killing.

"...exactly what effect the killing method has on sequence quality is not well known."

Freezing and killing with cyanide are also considered 'DNA-friendly', while some authors cautioned against the use of chemicals such as ethyl acetate or formaldehyde which were thought to degrade DNA.

In a recent study published in *Molecular Ecology Resources*, we examined the recovery and quality of DNA barcode sequences from South African Lepidoptera specimens collected using three commonly used killing methods (ethyl acetate, cyanide and freezing). We found that all specimens produced good quality DNA barcodes and there was no clear difference in nucleotide signal strength, probability of incorrect base calling and phylogenetic utility among the three treatment groups.

"...we examined the recovery and quality of DNA barcode sequences from South African Lepidoptera specimens collected using three common killing methods..."

“...ethyl acetate, cyanide and freezing can all be used to collect Lepidoptera for DNA barcoding studies.”

The sequence trace files of the three treatment groups all had high Phred quality scores (Figure 1) indicating that the data are reliable and there is very little background noise.

For more information about the results discussed in this article, see DOI: [10.1111/1755-0998.12331](https://doi.org/10.1111/1755-0998.12331)

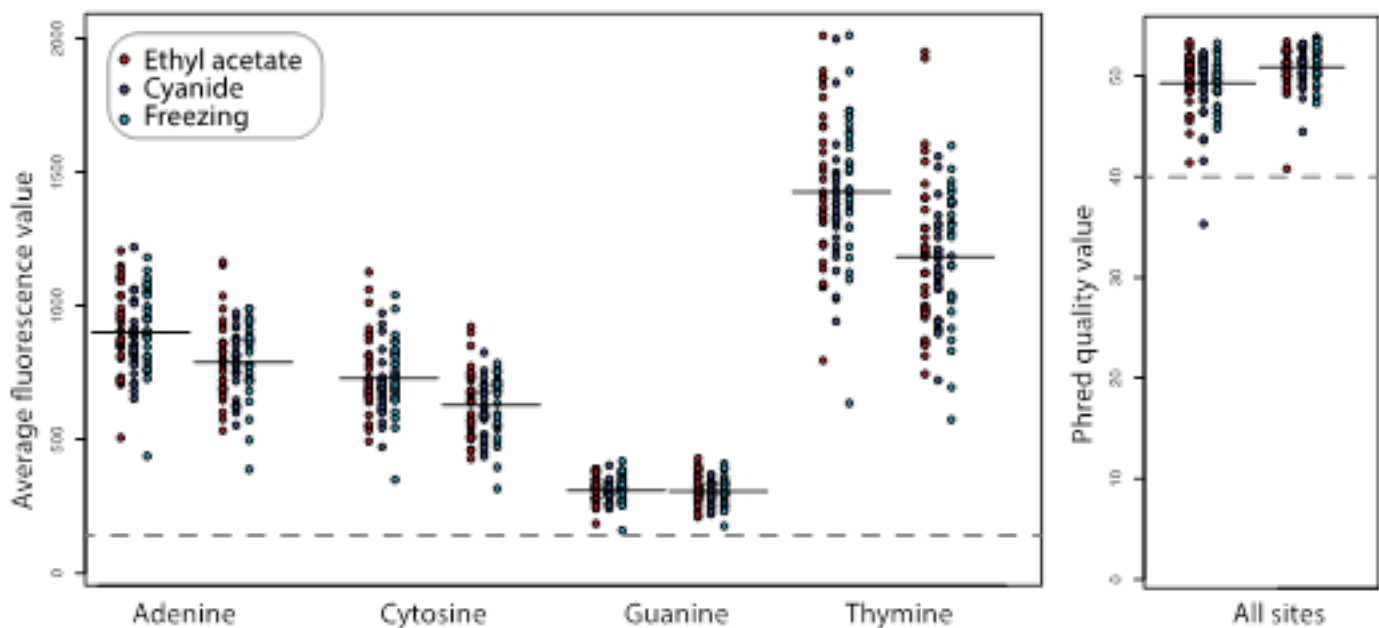
Phred scores recovered for both forward and reverse sequences were close to 50, indicating a base call accuracy of 99.99% or a probability of an incorrect base call of 1 in 100 000. This finding was quite surprising given that ethyl acetate has been reported to damage DNA even after brief exposure.

In summary, our study has shown that ethyl acetate, cyanide and freezing can all be used to collect Lepidoptera for DNA barcoding studies. Although we did not look at other insect groups it seems likely that the results from our study can be extrapolated to other arthropods.



Image credit: Alex Borisenko

Figure 1: Relative fluorescence unit (RFU) values for each nucleotide for both sequences generated using forward (LepF1) and reverse (LepR1) primers (panel on left). Phred quality scores for each sequence generated using forward and reverse primers (panel on right). Sequences generated from specimens collected using ethyl acetate, cyanide and freezing are displayed separately. In each case solid line represents mean value, and stippled line indicates accepted minimum thresholds (150 RFU and 40 Phred value). Figure taken from Willows-Munro & Schoeman *in press*.



Top 5 DNA Barcoding Publications 2014

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

1. Wells JD, Škaro V (2014) Application of DNA-based methods in forensic entomology in *Forensic DNA Applications: An Interdisciplinary Perspective*, ed. Dragan Primorac, Moses Schanfield, CRC Press, Boca Raton.
2. Bohmann K, Evans A, Gilbert MT, Carvalho GR, Creer S, Knapp M, Yu DW, de Bruyn M (2014) Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution* 29(6): 358-67. DOI: [10.1016/j.tree.2014.04.003](https://doi.org/10.1016/j.tree.2014.04.003)
3. Bocak L, Barton C, Rampton-Platt AC, Chesters D, Ahrens D, Vogler AP (2014) Building the Coleoptera tree-of-life for >8000 species: composition of public DNA data and fit with Linnaean classification. *Systematic Entomology* 39: 97-110. DOI: [10.1111/syen.12037](https://doi.org/10.1111/syen.12037)
4. Kelly RP, Port JA, Yamahara KM, Crowder LB (2014) Using environmental DNA to census marine fishes in a large mesocosm. *PLoS ONE* 9(1): e86175. DOI: [10.1371/journal.pone.0086175](https://doi.org/10.1371/journal.pone.0086175)
5. Wirta HK, Hebert PD, Kaartinen R, Prosser SW, Várkonyi G, Roslin T (2014) Complementary molecular information changes our perception of food web structure. *Proc Natl Acad Sci USA* 111(5): 1885-90. DOI: [10.1073/pnas.1316990111](https://doi.org/10.1073/pnas.1316990111)

Job Opportunity: Post Doc Position In Molecular Arctic Ecology

Applications are invited for a 2-year post doc (with a possibility of continuation) in the research group of Tomas Roslin at the University of Helsinki. The successful applicant will join the Spatial Foodweb Ecology Group, as funded by the Academy of Finland.

Starting date: Early 2015

Application deadline: 31 December 2014

The project is centered on the dissection of the high-arctic foodweb of Zackenberg, Northeast Greenland, by a range of techniques – many with a strong molecular component.

As an area characterized by low species richness, the High Arctic offers a unique opportunity for comprehensive assessments of food web structure, dynamics and functioning. Our work draws on six years of intensive field work as complemented by monitoring data generated over almost two decades by the Biobasis programme of Zackenberg.

This empirical fundament allows us to test influential ideas about how food webs are structured, and how they are

currently responding to changes in the arctic climate. We invite you to contribute to our exploration of this system – with your exact contribution developed on the basis of your own skill profile. By joining us, you will become an integrated part of our research team, together dissecting the structure and dynamics of arctic food webs.

To apply, you should have PhD / post doctoral experience with molecular ecology, and a strong interest in studying food webs by molecular approaches. Excellent written and verbal communication skills, the ability to think independently and creatively, and demonstrated team-working skills are musts.

Please email your application (CV with publications included, contact details of two references, and a letter (MAX 1 page) with a description of your research interests and why you would be a suitable candidate for the project) as a single pdf file to bess.hardwick@helsinki.fi

For informal inquiries, contact tomas.roslin@helsinki.fi
More information: <http://www.helsinki.fi/foodwebs/>

Credits and Contributions

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