

international
BARCODE
OF LIFE



Barcode BULLETIN

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The Ontario Bioblitz:
Exploring **Biodiversity**
and Connecting a
Community

Applications

Searching for
Substitutions
in a Natural
Sweetener

Research

Uncovering
the Truth
About the
Moa

Welcome to the **14th** issue of the Barcode Bulletin.

For the last thirteen issues the appearance of this newsletter hasn't changed much. After four years we thought it was time for a facelift. You will notice not only a change in layout in favour of a magazine look and feel but also the introduction of topical sections such as applications, research, education and more. This will allow readers to pick articles that they are most interested in for a first read and browse through the remaining ones. Each issue will also include a feature article we think will appeal to all our readers by highlighting special events or discoveries. For this issue we chose a report on a recent BioBlitz as these intense periods of biological surveying in an attempt to record all the living species within a designated area are becoming increasingly popular and more frequently incorporate DNA barcoding.

A lot of changes have come with this issue but, most importantly, what hasn't changed is the high quality of contributions by our peers. Let's not forget, most articles are written by researchers or enthusiasts, and not by professional writers, which makes our newsletter an even more impressive product. I'd like to take this opportunity to thank all of our contributors over the last four years. Most of the success of the Barcode Bulletin is owed to their efforts.

Dirk Steinke
Editor-in-chief

News Briefs

Planning is now underway for the 6th International Barcode of Life Conference which will be held from August 18th to 22nd, 2015 at the University of Guelph in Ontario, Canada. The conference will feature four full days of plenary and parallel sessions, a poster session, an evening keynote address, and multiple excursions to renowned Ontario destinations. A range of pre-conference workshops will also be offered to attendees. Additional details will be made available on the conference website, dnabarcodes2015.org, as they emerge.

Through a \$2.5 million project launched on the International Day of Biodiversity, researchers aim to enhance our understanding of Australian biodiversity using DNA barcoding. Andrew Lowe at the University of Adelaide will lead this highly collaborative project, which involves partnerships among numerous institutions: Kings Park Botanic Garden, CSIRO, Australian Tropical Herbarium, the South Australian Museum, ARC Centre of Excellence in Plant Energy Biology, and WA Department of Parks and Wildlife. Bioplatforms Australia, a research infrastructure organization, will manage the project and provide genomics and bioinformatics support. Specific research themes of this project include mapping biodiversity of plants and invertebrates for environmental impact assessments in mining and farming localities, promoting conservation of Australia's orchids, and combatting illegal logging as well as mislabeling in seafood markets.

An international group of researchers led by Marko Mutanen at the University of Oulu has received €570K from the Finnish Academy to explore several phenomena of barcode datasets. This research aims to better understand non-monophyly, deep splits, and barcode-sharing, among others, through genomic scans of selected target species using next-generation sequencing and methods such as RAD-Seq.

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Discoveries of the 2014 Ontario Bioblitz

Written by: Carlene Gallant (Biodiversity Institute of Ontario)

The Ontario Bioblitz was organized by the Toronto Zoo, the Royal Ontario Museum, Ontario Nature, Rouge Park, the Toronto & Region Conservation Authority and the Rouge Valley Conservation Centre. These collaborators recognized a unique opportunity to educate the growing population of this urban area, and introduce budding biologists to the exciting world of biodiversity.

With Parks Canada, the Biodiversity Institute of Ontario (BIO), and the Canadian Wildlife Federation as partners, the Ontario Bioblitz steering committee chose the Humber

watershed for its variety of natural and urban habitats. The 2014 blitz was hosted by the McMichael Art Gallery and the Kortright

Centre for Conservation in Vaughan. The Humber watershed is the largest in the Toronto region and, as a protected Canadian Heritage Site, it provides an ideal location for residents to learn more about Ontario's biodiversity.

The event started with the opening ceremonies at noon, and then the blitzing began! By definition, a Bioblitz is a concentrated effort of naturalists, biologists and enthusiasts of varying degrees of expertise, to identify and record as many species as possible. It is confined to a given area and timeframe - for the Ontario blitz, a 24 hour period.

Throughout the Humber watershed, several sampling locations were chosen based on different vegetation types and habitats. BIO played a large role in sampling, especially the invertebrate collecting teams. Teams began by setting up standard numbers and types of traps in select sites. When this was complete, the teams began exploring, some in search of a specific taxon, others looking for any organism that crossed their path.



"...a unique opportunity to educate the growing population..."

When dusk approached, participants headed over to the McMichael Art Gallery to sort and identify their material. This continued until blitzers were unable to keep their eyes open and the night closed with the group curling up in a cluster of tents on the Gallery grounds. They awoke early the next morning to finish up collecting and take down traps; it then became a mad rush to database the final specimens and organize species counts.

"...some in search of a specific taxon, others looking for any organism that crossed their path."

The total species count for this year's blitz was 1563 and rising! Over 700 of these included invertebrates, some of which were not previously known to the area. *Pholcus opilionoides* is a spider which was known to Europe but is a new addition to the Canadian fauna and reported as an introduced species.

In addition to focused sampling by scientists, this year offered guided blitzes for amateurs to learn collecting and identifying techniques. Several trained interpreters were selected to guide a group through the basics of sampling a general taxon. BIO led some invertebrate guided blitzes, including a terrestrial and nocturnal event. Based on the experience of the group leaders and the attitude of the participants, this new idea was a huge success. Participants were wildly enthusiastic and came from all age groups, interests, and experience levels. Despite the variety of backgrounds throughout the groups, they shared an overwhelming dedication to learning about the abundance of life around them.

Next year's Bioblitz is expected to be even more spectacular and there are discussions underway to have it coincide with the 6th International Barcode of Life Conference. To learn more, check out www.ontariobioblitz.ca.

Thank you Vincent Luk for providing photos and capturing the enthusiasm and hard work of all the Bioblitz participants.





MadLab, DIYbio and the Riddle of the “Mystery Meat”

Written by: Asa Calow (MadLab, Manchester UK)

At MadLab, we describe ourselves as a community space for science, technology and art. What started out in 2009 as a physical manifestation of several online communities (mailing lists, blogging circles and the like) has developed into a busy creative space hosting all manner of groups – from amateur electronics to jewellery, fiction writers to app developers. As the interest in DIY and making/hacking culture has risen in the past few years, MadLab has grown with it – from six groups to more than 50, sprouting countless workshops and projects in turn.

Back in 2010, thanks to a chance encounter at one of MadLab’s book clubs, a partnership was formed with Manchester Metropolitan University’s NanoInfoBio project. Our area of common interest was the then-emerging phenomenon of “do-it-yourself” biotechnology (DIYbio). The partnership was a fruitful one, and has led in turn to a four-year mission of amateur scientific and technical discovery.

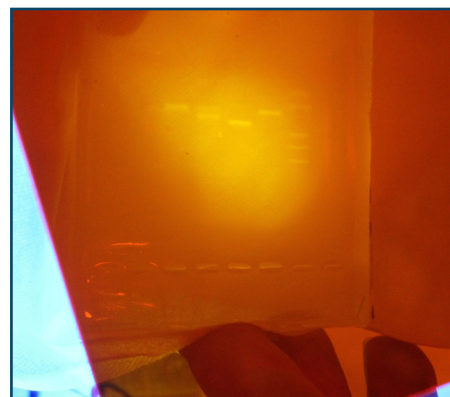
This brings us to the present day.

We knew that others in the global DIYbio community had DNA barcoding know-how – Genspace in NYC were part of the Urban Barcode Project team, for instance.

Closer to home, in the wake of the horse meat “food fraud” scandal, Thomas Landrain from Parisian “biohackspace” La Paillasse had crafted an intriguing DIY-friendly procedure which we felt we could adapt to our needs.

So we hopped on a plane, to spend a day with Thomas in a breeze block basement on the city’s outskirts, returning home with a mild pastis hangover along with the practical knowledge needed.

“...a busy creative space hosting all manner of groups...”



The final gel. Note the double stripe in lane two, our lamb mince has a suspected secret ingredient...

The next challenge was building a lab. MadLab isn't a traditional science space, and we aren't resourced in a way which allows us to drop lots of money on expensive equipment. Fortunately, we have a community of engineers and designers on hand who are well versed in the art of "frugal innovation" (or jugaad) to help get around this.

Another stroke of luck was the arrival of itinerant DIYbioscientist Noah Most from the US – you can read about his exploits visiting global biohackspaces in Biocoder magazine. Noah spent a month with us, and in that time we built from junk and borrowed (thanks again MMU!) the equipment we needed.

"...for us, "mysterious meat" is just the beginning."

Finally the day came to run our experiment.

We gathered an interested group together and, after a quick trip to the local butchers for samples, got to work. The three protocol steps – DNA extraction, amplification and visualisation – worked admirably on our DIY kit, and showed up some intriguing results.

Of particular interest was the sample labelled "100% lamb mince", which presented itself as containing a significant quantity of beef. Needless to say, we'll be back for more soon.

So for us, "mysterious meat" is just the beginning. We have already begun looking at other barcoding protocols, and there are plans for more investigations on the horizon – at this year's Manchester Science Festival, and in the nearby woodlands of Boggart Hole Clough. Stay tuned!



The DIYbio laboratory in action.



DIYbioscientist Noah Most busy with protocol practice.

Barcoding the Trendy Sweetener Stevia

Written by: Lucía Gato-Calvo, Joaquín Vierna (AllGenetics), and Hugo de Boer (Uppsala University)

'Natural', additive-free products have become increasingly popular among health-conscious consumers in recent years. The current worldwide obesity epidemic, combined with a growing aversion to the perceived multitude of synthetic ingredients that we ingest with a variety of foods and beverages every day, has contributed to the appearance of a booming market for products marketed as 'natural' or 'organic'.

Sugars such as glucose and high-fructose corn syrup are considered unhealthy, and although artificial sweeteners such as aspartame, sucralose, neotame, and saccharin are commonly used, people would generally prefer natural substances.

One of the most commonly used natural sweeteners is Stevia from *Stevia rebaudiana* (Bertoni) Bertoni. Following US Food and Drug Administration approval of Stevia as a food

additive in 2008 and EU Food Safety Authority approval in 2011, the number of products containing it has skyrocketed.

Stevia Cav. is native to the Americas, and currently there are 261 accepted names in the genus. Most research has focused on *S. rebaudiana* and it is the main source of steviol glycosides.

"...a booming market for products marketed as 'natural' or 'organic'."

This herb is now widely cultivated to meet the growing commercial demand for it. Although the species may have been used by Guarani Indians already since ancient times, its contemporary use dates only from the 1970s. Stevia is available in many different forms, such as purified extracts of rebaudioside A, dried leaves, powders, tablets, and tea bags.

Our pilot study was a collaborative project with AllGenetics, a commercial genetics lab based in Spain, and aimed to apply DNA barcoding to selected Stevia herbal teas from different suppliers. Stevia leaf fragments were carefully examined using stereo microscopy, and six fragments from each preparation were selected for DNA barcoding using the nrITS barcoding region. The 24 sequences obtained were compared with Stevia nrITS sequences available in NCBI GenBank (95 sequences from 75 Stevia species) using both a RAxML phylogenetic framework and BLAST.

The sequence analyses showed that all Stevia samples contained the intended Stevia species *S. rebaudiana* exclusively.

Some of the material in the Stevia samples came from other genera, and three sequences from *Cynodon dactylon* (L.) Pers., *Digitaria* sp., and *Cenchrus* sp. were identified. The lack of Stevia species substitution is probably due to the fact that virtually all Stevia is cultivated, and that only *S. rebaudiana* is known to contain steviol glycosides. The detection of other species in the tested preparations can be ascribed to accidental additions from the harvesting process or possibly to the intentional addition of 'fillers'.

Commercial DNA barcoding for species authentication in herbal supplements is both powerful and cost-effective, and is likely to become increasingly important for quality control. The lack of species substitution detected in this study will be reassuring to consumers interested in sugar-free 'healthy' and 'natural' products containing Stevia preparations.

"Commercial DNA barcoding for species authentication in herbal supplements is both powerful and cost-effective..."



Image credit: flickr.com/photos/h-bomb/



Image credit: flickr.com/photos/healthgauge/



Image credit: flickr.com/photos/jeepersmedia/

The three basic moa shapes: large and quasi-cursorial; medium-sized and extremely graviportal; small to medium-sized and cursorial.



New Zealand's Extinct Moa: How Many Species Were There?

Written by: Leon Huynen, David Lambert (Griffith University), and Craig Millar (University of Auckland)

The number of species of New Zealand's extinct flightless moa remains unclear despite over 150 years of research. Early studies based on bone morphology suggested the number of species may have been well over 30. Later, upon reanalysis and a relaxation of the morphological limits of each species, this number was reduced to 13.

The development of ancient DNA technologies allowed, for the first time, a molecular approach to the problem. Using mitochondrial control region sequences and sex-specific genetic markers, a number of moa species morphologically similar but different in size, were shown to be males and females of the same species. As a result moa were shown to exhibit an extreme form of reverse sexual dimorphism, with the females of some species being over twice the weight of their male counterparts.

"The development of ancient DNA technologies allowed, for the first time, a molecular approach to the problem."

Although analysis of mitochondrial control region sequences reduced the number of some species, the sequence data also suggested the existence of several new clades within the genera *Dinornis* and *Megalapteryx*. Using the COI barcoding region to try and finally resolve the number of moa species, we barcoded 38 bone samples representing most control region variants.

The barcoding results suggested two species for *Dinornis*, the North and South Island giant moa; one mid-sized species, the Little bush moa, present on both New Zealand's North and South Island; and one large species, the Eastern moa, restricted to New Zealand's South Island. Furthermore, within the genus *Pachyornis* one new species was suggested in addition to the three currently recognised species. This new species is a large heavy bird with individuals being restricted to the South Island of New Zealand.

The species status of individuals belonging to the genus *Euryapteryx* is more complex, with COI data suggesting two species, one at each end of New Zealand, and a possible mix of *Euryapteryx* populations occupying New Zealand's central regions.

The species status of the remaining moa genus *Megalapteryx*, remains unresolved due to a lack of COI data. Control region sequences however suggest that *Megalapteryx* can be grouped into four distinct clades.

To date, barcoding analysis suggests the existence of at least 11 species of moa, with possible further species yet to be identified in the genus *Megalapteryx*. COI barcoding has in the case of moa proved invaluable for the identification and classification of this extinct species, particularly in the absence of substantial morphological, behavioural, and physiological data.

“...barcoding analysis suggests the existence of at least 11 species of moa...”



Moa excavation at Kapua Swamp in New Zealand's South Island. A large pile of moa leg bones can be seen in the top right of the picture.



'The arrival of the Maories'. Early cartoon by Trevor Lloyd (1863-1937) depicting the arrival of Polynesians to New Zealand in the 13th Century.



Species Assessment in African Orchid Cake

Written by: Sarina Veldman, Hugo de Boer, Joseph Otieno (Uppsala University),
and Barbara Gravendeel (University of Applied Sciences Leiden and Naturalis Biodiversity Center)

Chikanda is a traditional snack made of boiled terrestrial orchid tubers and peanuts, which is popular in Zambia and Southwestern Tanzania. In recent years *chikanda* has become increasingly popular, and this has fueled a flourishing tuber trade. The Zambian market is far larger than the Tanzanian, but Zambian orchids alone are insufficient to meet the skyrocketing demand, and tubers are now imported from surrounding countries such as Tanzania, Malawi and Angola.

Tubers used for *chikanda* are mainly derived from three terrestrial orchid genera: *Disa*, *Satyrium* and *Habenaria*. Tubers are harvested unsustainably and species seem to be targeted indiscriminately. In Tanzania this puts as many as 85 terrestrial orchid species at risk of overharvesting, some of which are locally common and others that are rare endemics. A challenge to orchid conservation is that it is not known which

“Tubers are harvested unsustainably and species seem to be targeted indiscriminately.”

species are used for *chikanda* exactly. Tubers traded in the market are unidentifiable up to species level using morphology alone, and until now identifications have been based on field collections made during the harvesting season, or by propagation of the tubers until flowering.

These methods are very time consuming, prone to introduce uncertainties, and do not allow species assessment of the end product, the *chikanda* itself.

In our project we are using an IonTorrent PGM for Next Generation Sequencing to analyze the species content of 6 prepared orchid cake, *chikanda*, samples, bought on markets at either side of the TZ-ZA Tunduma border. We are using two fragments of the nuclear internal transcribed spacer: nrITS1 and nrITS2, as barcoding markers. Generated amplicons (>200bp) were matched using BLAST against reference sequences on NCBI GenBank for preliminary identification.

Results show that the presence of *Disa*, *Satyrium* and *Habenaria* species could be confirmed up to genus level, and also confirm the presence of peanuts (*Arachis hypogaea*) and chili pepper (*Capsicum* spp.). Other sequences resulting from contaminants or possible adulterants were also found, e.g. sweet potato (*Ipomoea batatas*), mango (*Mangifera indica*) and pumpkins (*Cucurbita* spp.).

Accurate species level identification of the orchids present in *chikanda* is partly due to restrictions in the number of reference sequences in GenBank. Another problem can be limited variation in barcoding markers between closely related species, such as we found in ITS for the genus *Disa*. In the case of *Satyrium* some

Species-level identifications require “extensive field collection to increase reference coverage of the many rare endemics.”

sequence clusters generated a single match, indicating the presence of, amongst others, *Satyrium trinerve*, *Satyrium shihense*, *Satyrium volkensii* and *Satyrium breve*.

Although none of these species occur on IUCN red-lists, the presence of these orchids in *chikanda*, combined with high tuber demand, give an indication of possible susceptibility to overharvesting.

Overall it seems that Next Generation Sequencing is very useful in genus-level assessment of ingredients used for *chikanda*. However, for identification up to species level complete reference databases of putative species are necessary. In the case of African orchids this requires extensive field collection to increase reference coverage of the many rare endemics.



Image credit: Barbara Gravendeel



Image credit: Sarina Veldman



Understanding Community Dynamics Through DNA Barcoding

Written by: Gillian Martin (University of Guelph)

While DNA barcoding has improved our ability to capture species diversity, few studies have sought to determine how a better description of community composition affects our understanding of community dynamics. This is particularly interesting for invertebrate communities where species can be difficult to identify.

To assess the impact of taxonomic resolution, we need to understand how one community can differ from another. Community formation is affected by neutral dynamics (all species are ecologically equivalent) and niche dynamics (species have specific environmental preferences). In a metacommunity (a set of possibly interacting communities), the species dispersal ability also impacts what community a species may colonize.

However, very few studies have looked at the effect of taxonomic resolution on metacommunity models or incorporated DNA barcoding techniques. We conducted a study to determine

whether there is a relationship between taxonomic resolution and metacommunity model explanatory power.

In mid-summer of 2011, we sampled the aquatic invertebrate communities of 21 streams in Algonquin Provincial Park, Ontario, Canada. Following standardized biomonitoring protocols, we randomly collected a minimum of 300 aquatic invertebrates, and measured 23 environmental variables at each stream. For this study we focused our sequencing efforts on the six most ubiquitous invertebrate orders (Coleoptera, Diptera, Ephemeroptera, Oligochaeta, Plecoptera, Trichoptera).

Based on our abundance data, we created 3 site-by-species matrices for each order: a family level, a genus level, and a species-level based on Barcode Index Numbers (BINs). To determine the relative roles environment and space played in structuring the communities we used variation decomposition, which breaks down

the variance in species data into proportions explainable by environment, space, and the interaction between environment and space.

To facilitate comparisons across orders where evolutionary timescales may impact what a genus or family level grouping means, we calculated mean-interspecific sequence divergence using Kimura 2-parameter analysis tools on the Barcode of Life Data Systems.

We found that there was a significant relationship between taxonomic resolution and metacommunity model explanatory power. As taxonomic resolution decreased (species to family), the amount of variation explained by the environment increased by up to a difference of 26.3%. Spatial variables were only significantly related to the assemblage structure for Diptera and Oligochaeta communities at a BIN level and explained less than 7% of the variation. This decrease is likely because closely related species may share more similar traits; therefore, grouping species together into genera or families could result in a stronger relationship between community composition and environment.

“...there was a significant relationship between taxonomic resolution and metacommunity model explanatory power.”

The notion of neutral species within a genus lowering the detectability of the environmental signal has not received a lot of attention, though there is some indirect evidence for aquatic insects based on similarities in niche requirements. Our results suggest that there could be biologically cryptic species present in the groups studied, which are not affected by the explanatory variables measured, either due to neutrality or factors that affect the terrestrial adults.

By more accurately capturing species composition, DNA barcoding provided a method to better assess the impact that taxonomic resolution has on our description of metacommunity dynamics.





DNA Barcoding the Bats of Malaysia

Written by: Khairunnisa Syaripuddin, Kong-Wah Sing, and John-James Wilson (University of Malaya)

Image credit: Khairunnisa Syaripuddin

Bats are important both ecologically and economically; controlling insect populations and as pollinators and seed dispersal agents. The faecal matter of insectivorous bats is a potent fertilizer used by the agricultural industry as well as the primary nutrient source in cave ecosystems. Ecotourism, focused on bat populations, can boost national economies, providing social and economic benefits to local people in host communities, while also promoting bat conservation.

The human interest in bats, coupled with their unique biology, suggests bats could be useful, yet currently underappreciated, models for ecology particularly as surrogates for “total” biodiversity and as indicators of environmental contamination resulting from changes in land use.

More than 10% of the bat species of the world can be found in Malaysia. Krau Wildlife Reserve is home to at least 51 bat species and holds the record for the single site in the old world with the highest bat species richness following intensive surveys during the early 2000s.

“More than 10% of the bat species of the world can be found in Malaysia.”

However, the species richness of bats across Southeast Asia might be underestimated by at least 50%.

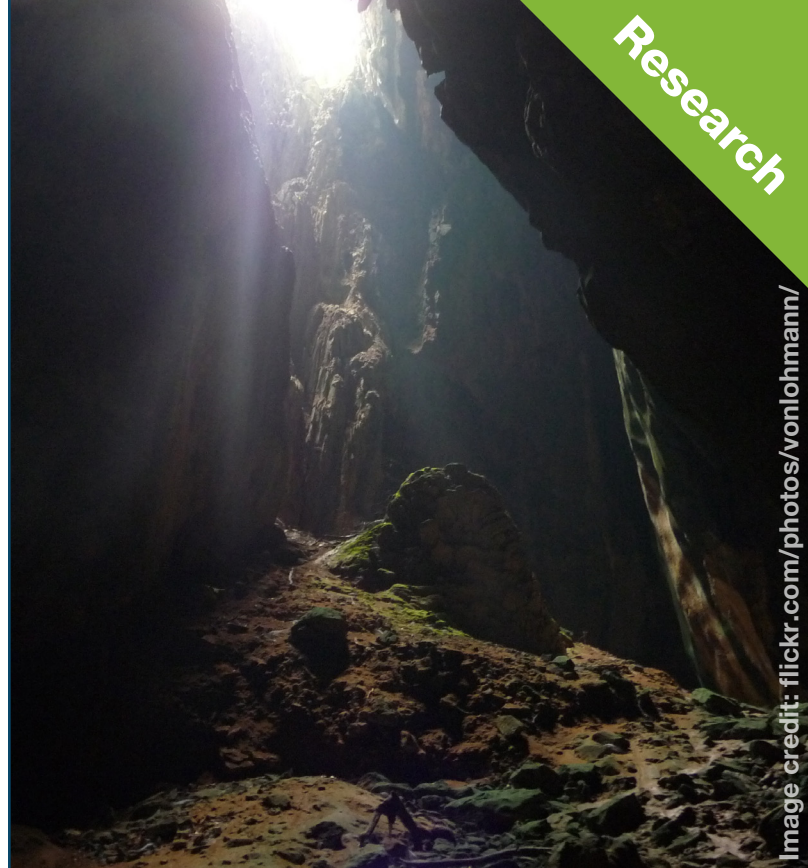
An extensive study by Charles Francis and colleagues, incorporating DNA barcoding, discovered much higher levels of endemism and greater intra-specific population structure in Southeast Asian bats than had previously been recognised. The high number of overlooked species could be attributable to the cryptic behaviour and morphology of bats.

Many of these cryptic bats recognisable through DNA barcoding currently have informal names like *Hipposideros bicolor*¹³¹ or *Cynopterus* cf. *brachyotis* Forest. They are so-called “dark taxa”. However, we suggest this should not be a major worry or impediment to those interested in studying bats.

Hipposideros bicolor is one of the many species split into two through DNA barcoding.

Our own surveys of bats at sites across Peninsular Malaysia shows these names can be used exactly like traditional names - connecting individuals of the same species across surveys and providing anchor points for biological data associated with individuals and species.

The situation with Malaysian bats shows that although a species name obtained from BOLD might not always be in step with Linnaean style species names this needn't hinder progress in ecological research.



The Dark Cave at Batu Caves is a famous ecotourism destination in Malaysia. Bat guano is the primary nutrient source for the cave food webs that support species found nowhere else.



DNA Barcoding Enhances Vegetation Surveys

Written by: Ken Thompson (University of Guelph)

Vegetation surveys are conducted to obtain a catalogue of the plant species that occupy an area of interest, and are used to detect and monitor areas that require conservation. Typically, surveyors identify collected specimens using morphological features—most often floral structures. Because plants flower at different times throughout the growing season, survey sites must be revisited repeatedly to obtain a complete inventory, which can add considerably to the associated costs. In addition, identifying plants using morphology is challenging, and many specimens collected in vegetation surveys are not identified to the level of species.

With molecular taxonomic tools such as DNA barcoding, only a small section of leaf tissue is required to identify a specimen. This lack of a need to collect specimens when they are flowering enables surveyors to obtain a complete catalogue of an area's resident flora in a single

visit, which may reduce costs by lessening the amount of time spent in the field. In addition, DNA barcoding may allow surveyors to conduct more accurate surveys since plant identification skills are not required to identify collected specimens if there is a comprehensive barcode reference library available.

“At present, the demand for species conservation far exceeds the available funds.”

To determine if DNA barcoding can lessen the costs of vegetation surveys whilst increasing their accuracy, we conducted two vegetation surveys in northeastern Ontario—we used traditional methods of identifying species using morphological features in one survey, and we used DNA barcodes to identify collected specimens in the other. We sampled the same 337 forest plots in both surveys, and recorded all flowering plants, ferns, mosses, and lichens present in each plot.

We found that the survey conducted using DNA barcoding was 37% less expensive than the survey conducted using morphology for species identification. While identifying collected specimens using DNA barcoding was more expensive than using morphology, the increased time required in the field when morphology was used for identification resulted in the

“...surveys conducted using DNA barcoding outperform traditional morphology-based methods...”

DNA barcoding identified 2.6 more species per plot relative to the morphology-based survey and 60 additional species in total, indicating that DNA barcoding improves the accuracy of species richness estimates.

morphology-based survey having a higher total cost than the survey that used DNA barcoding.

In addition, we found that the survey conducted using

At present, the demand for species conservation far exceeds the available funds. With this in mind, practices that improve efficiency in the use of funding, as well as ensure its optimal allocation, are of fundamental importance.

Our study demonstrates that vegetation surveys conducted using DNA barcoding outperform traditional morphology-based methods in both of these areas. First, by decreasing the associated costs of vegetation surveys, more surveys can be conducted for a fixed cost if DNA barcoding is used rather than morphology. Second, our result that DNA barcoding improves the accuracy of species richness estimates can lead to more appropriate allocation of conservation funding. We conclude that greater effort should be made to implement molecular taxonomic tools like DNA barcoding for species identification in vegetation surveys.



Barcoding Proturans of Austria

Written by: Nikolaus Szucsich (University of Vienna)

If staying enigmatic would warrant evolutionary success, they would be among the most successful animal groups on Earth; Protura, also referred to as coneheads, are a group of primarily wingless insects. First described just over a century ago, a concealed distribution of morphological diversity motivated only a handful of committed taxonomists to carve out phylogenetic signal from cryptic characters.

Our recent study thus aimed to test the accuracy of these inconspicuous diagnostic characters by a barcoding approach based on two independent markers: the standard barcoding fragment and

“...coneheads are among the record holders of within-species variability.”

a fragment of the nuclear 28S rDNA gene after Non-Destructive DNA-Extraction. Not only is the signal from both markers fully congruent with the current taxonomy,

we likewise were able to characterize a species new to science with both morphological and molecular characters.

The most interesting outcome, however, lies in the distances among different populations of what is currently assessed to be the same species. Not surprisingly, populations of proturan species can be differentiated by both markers, given that the dispersal ability of these animals is highly limited by both their small size

(with body lengths of 1mm and below) and their hidden life style in the soil. With intraspecific distances of up to 21.3%, coneheads are among the record holders of within-species variability. This is even more remarkable considering that the three populations chosen for this study by far do not cover the distributional range of the studied species.

Two of the most important lessons we can learn from coneheads are: Firstly, even a high mismatch regarding observable diversity between morphological and molecular data does not necessarily result in incongruent phylogenetic signal from these data sets. Secondly, the restriction of biodiversity to species numbers is highly prone to underestimations, especially in organisms with restricted dispersal ability.

Future work will focus on some important points: (i) The protocol will be improved to allow for a more standardized barcoding approach for Protura. (ii) The range of sampled localities will be widened, both to cover more species and a higher number of populations within each species. (iii) The results will be compared to other groups of soil-living arthropods. All of these studies will be incorporated into the newly launched ABOL-Initiative (*Austrian Barcode of Life*).

Insect Diversity of San Diego County's Biodiversity Hotspot

Written by: Dirk Steinke (Biodiversity Institute of Ontario) and Bradley Zlotnick, MD (San Diego Barcode of Life)

Summer 2013 marked the establishment of an international research project exploring insect diversity across the San Dieguito River Valley, a 55-mile conserved transect of the global biodiversity hotspot that is San Diego County, California. A unique collaboration of the University of California, San Diego (UCSD), the San Diego Zoo Institute for Conservation Research (ICR), San Dieguito River Park and Conservancy (SDRP), and the Biodiversity Institute of Ontario, this Malaise trap study is the first of its type in Southern California, and perhaps the entire USA.

The SDRP project aims to acquire and evaluate detailed temporal and spatial information on terrestrial arthropod communities in this region as the focal point of a larger San Diego Barcode of Life (SDBOL) initiative. With a view to BIO's Global Malaise Project and School Malaise Trap Program, the SDRV group's quarterly collections use these very effective sampling devices for many groups of insects, with subsequent DNA barcode analysis of specimens at BIO. The sequence data assign individuals from each trap to BINs, enabling the quantification of both alpha and beta diversity patterns, with

BINs serving as a proxy for species counts. As with any other collection method, Malaise traps capture only a segment of the total insect fauna, though they have the advantage of being easier to deploy in a standardized way than other collection methods and of being cost-effective in terms of specimens gathered per collector-hour.

No more eye-burning hours, gazing down microscopes.

Six Malaise trap sites are in place across the 120,000 acre San Dieguito River Valley watershed, each in one of the County's most important habitat types, from coastal estuaries through chaparral to 5000 foot inland ranges. Traps are deployed and maintained by UCSD, ZooICR, and SDRP staff and UCSD students. First year collections have been built around quarterly two-week sampling, including a trial of local pre-sequencing sorting at the Zoo ICR facility. While planning to extend the sampling protocol in to future years, potentially with increased frequency and breadth, the SDBOL group is stimulating participation by other local conservancies, corporate sponsors, and individuals' sites.

The first six Malaise trap samples collected in two weeks in August 2013 were analyzed in the fall. They contained a total of some 5400 specimens of which about 4100 were subsequently barcoded.

The result – 845 BINs with over half of the insect specimens being flies (Diptera), followed in abundance by moths and butterflies (Lepidoptera), bees, ants and wasps (Hymenoptera), beetles (Coleoptera), and true bugs (Hemiptera). Supplementing large in-kind efforts by the founders, the Consulate of Canada in San Diego provided critical seed funding for sequencing.

These pilot results are just a snapshot – six trap-weeks out of forty eight for the year. This first dry-season collection might be expected to expose lower levels of diversity.

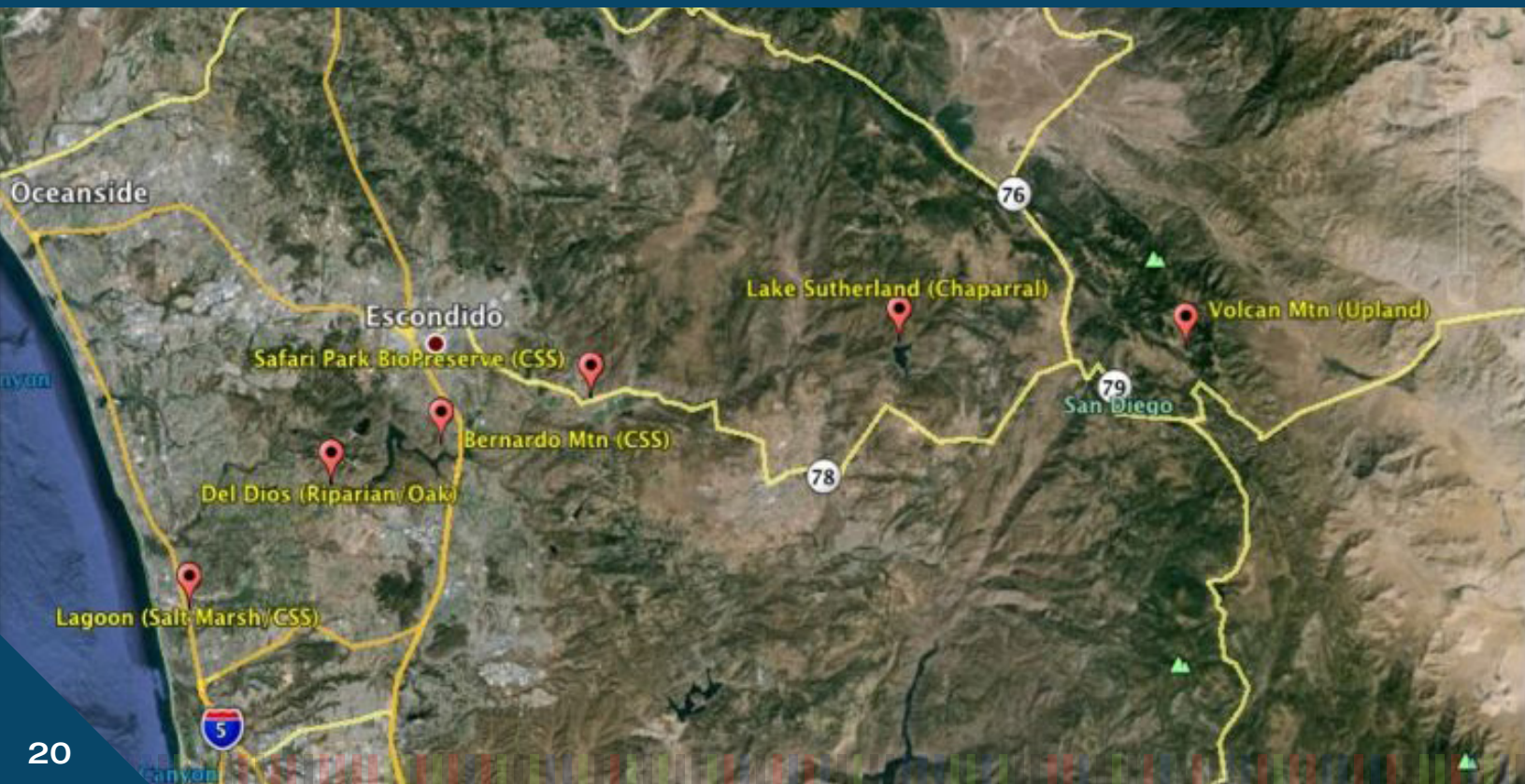
As traps go out in August for the 2014-15 project year, we will be completing the remainder of the 2013-14 sequencing looking to refine SDRP accumulation curves which suggest estimates of total insect diversity exceeding several thousands of species.



Image credit: David O'Connor

Malaise trap at San Diego BioBlitz 2014.

Six Malaise trap sites are in place across the 120,000 acre San Dieguito River Valley watershed.



"...we were happy to be part of something exciting and it made the learning we did more fun."

Education



Image credit: Tyler Schaefer

The School Malaise Trap Program: A Student Perspective

Written by: Aric Brewer and Hunter Rose

This article was written by two grade 6 students at Listowel Central Public School in Listowel, Ontario, Canada, who specifically oversaw the tracking and daily supervision of their school's Malaise trap for the Fall 2013 School Malaise Trap Program.

Listowel Central's grade 6 class was one of many classes which were excited to experience some real learning in action.

Our teacher, Mr Schaefer, found a backyard that was green, moist and surrounded by trees. It is important to be in that environment because bugs thrive in a vegetated atmosphere. Our class set up the trap which was like a small tent with a bottle of ethanol. We observed the bottle and tent three to five times a week to make sure the tent wasn't damaged and we checked the bottle to estimate and look for different kinds of bugs.

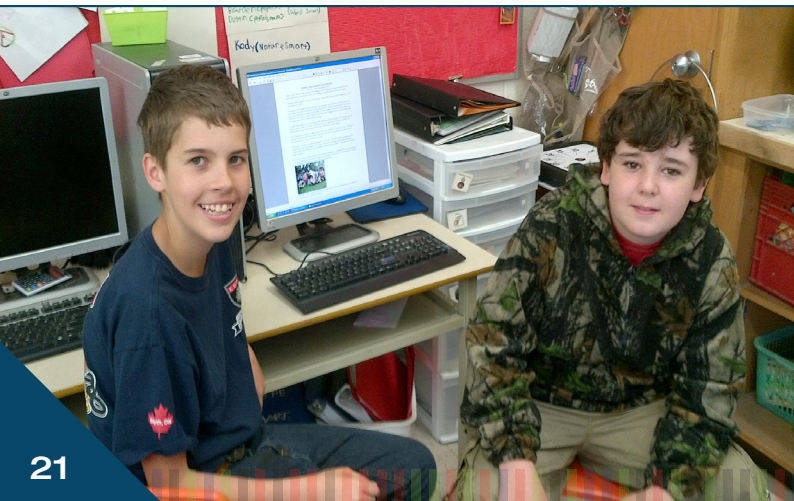
Visually there appeared to be less bugs in the bottle than what we thought. We estimated about sixty to seventy bugs in a bottle. Ultimately it was more than three hundred in a bottle. We viewed various bugs in our bottles, such as flies, bees, spiders, and earwigs.

The Biodiversity Institute of Ontario shared with us the results of our Malaise Trap. We learned that the bug that was the most common were the flies and the bug that was the least common was the lacewings. We also found 6 new bugs.

"We stared in wonder at all the detail of the small living things."

We found it exciting to see the photographs of the insects. We stared in wonder at all the detail of the small living things. The species we were most intrigued with were the ground beetle, plant bug, leafhopper, trichogrammatid wasp, braconid parasitic wasp and an unidentified hymenopteran.

In the end, we were able to monitor a true science experiment. If nothing else, we were happy to be part of something exciting and it made the learning we did more fun. We would like to thank the Biodiversity Institute of Ontario for this opportunity.

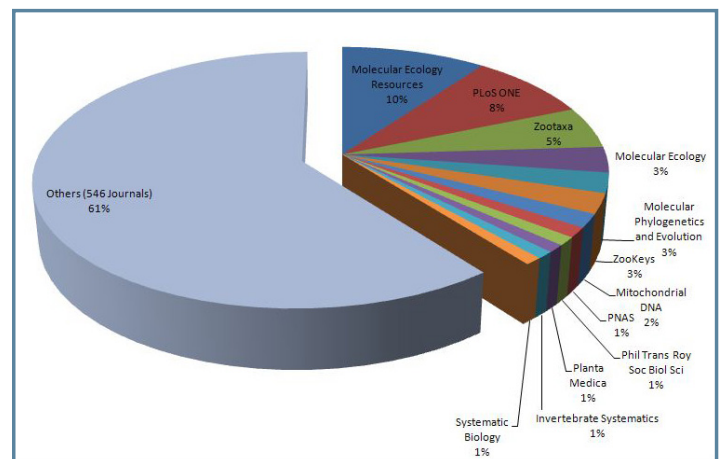
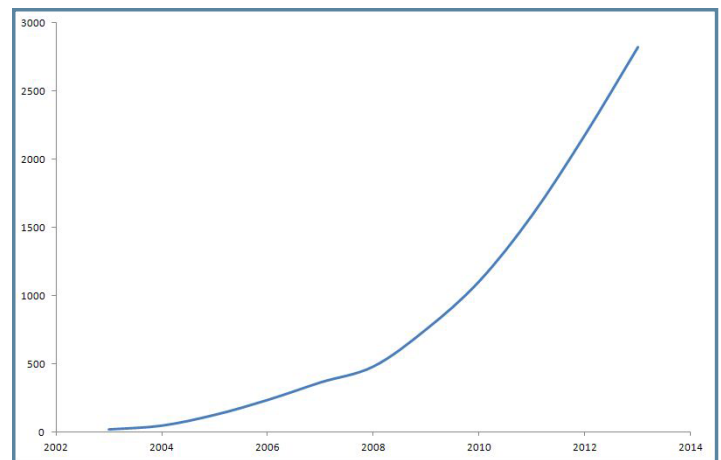


Trends in DNA Barcoding Publications

Written by: Dirk Steinke (Biodiversity Institute of Ontario)

In the last issue of the Bulletin we showed a figure of the steady growth in numbers of DNA barcoding publications over the last 11 years. Since the publication of the famous Hebert *et al.* paper in 2003, some 3000 publications involving DNA barcoding have been produced. The first here shows this using the cumulative numbers over the years. Essentially it represents an accumulation curve of DNA barcoding publications and as with most species accumulation curves we are a long way away from saturation.

“...558 different journals have published at least one DNA barcoding paper.”



The second figure demonstrates how diverse the field has become as it depicts the number of journals that have published DNA barcoding papers and shows preferences among authors. In total 558 different journals have published at least one DNA barcoding paper. About 260 did more than that. Two journals are in the lead which is the result of their efforts to become home for DNA barcoding research publications. Molecular Ecology Resources has a strong focus and PLoS ONE with its inclusive concept is catching up quickly. The third place is another very good indicator of how DNA barcoding has impacted taxonomic research. Zootaxa is the world leading journal on taxonomy and main home for species descriptions and revisions. However, ZooKeys, their open access competitor is catching up quickly.

Unfortunately, publications on DNA barcoding follow the general trend that favors journals behind a paywall. Only 16% of the papers are OpenAccess.

Credits and Contributions

Editors: Dirk Steinke
Emily Berzitis
Design: Suz Bateson

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