

international
BARCODE
OF LIFE



Barcode BULLETIN

June 2015
Vol.6, Issue 2

Barcoding **Feather Mites:** Addressing a **Challenge** for **Evolutionary Ecology**

Applications

Investigating
Diet Diversity
of Beetles in
Steppic Habitats

News

6th International
Barcode of Life
Conference

Welcome to the June 2015 issue of the iBOL Barcode Bulletin.

This is our last issue before the 6th International Barcode of Life Conference here in Guelph. However, we are planning a special issue of the Barcode Bulletin with contributions from conference participants. This special feature will be available on August 18th, the first day of the conference.

This issue of the Barcode Bulletin features stories and studies about feather mites, mosquitoes, tarantulas, beetles, pencilfish, earthworms, as well as gastropods from an island with a dubious past. We will also have a look at herbal supplements and college student projects that involve DNA barcoding and birds.

Dirk Steinke
Editor-in-chief

School Contest Winners

Participants of the **School Malaise Trap Program** were invited to submit photos of their classes with the Malaise trap for the #SMTPselfie contest. Five winners were selected and each one received a **LifeScanner kit**.



M.C. Knoll Elementary School



John Polanyi Collegiate Institute



Iona Academy



Chedabucto Education Centre



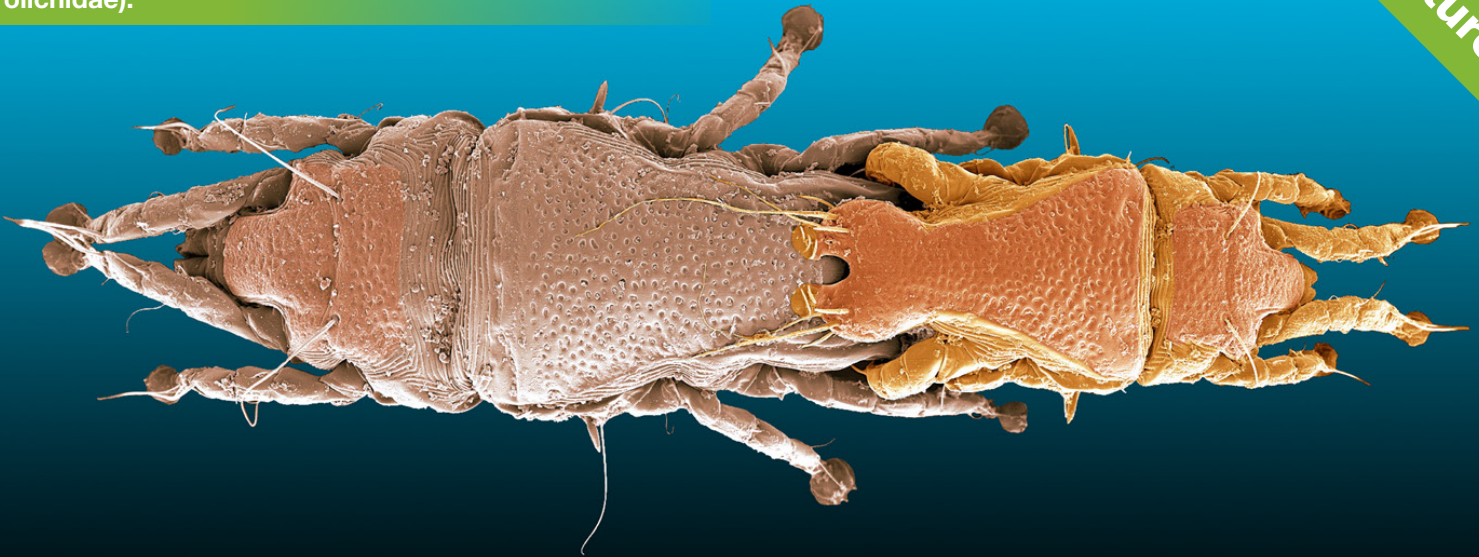
École Précieux-Sang

News Brief

The Biodiversity Institute of Ontario recently received \$3.55 million CAD in funding through the Ontario Research Fund's Research Excellence program. These funds will help BIO to further expand the DNA barcode reference library and to support growth of the Barcode of Life Data Systems.

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0.1 mm

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Photo © Dave Walter (University of Alberta)

Barcoding Feather Mites

Written by: Jorge Doña, Javier Díaz-Real, Pilar Bazaga, David Serrano, Roger Jovani
(Estacion Biológica de Doñana (CSIC), Spain) and Sergey Mironov (Universitetskaya Embankment 1, Russia)

Feather mites are among the most abundant ectosymbionts associated with birds. They live permanently on birds and are transmitted among them mainly by direct bird-to-bird body contact (e.g. from parents to offspring in the nest).

Within feather mites some families belonging to Analgoidea are inhabitants of the skin and nasal cavities of their avian hosts. These mites seem to be true parasites of birds. However, most feather mites are highly specialized dwellers of different

How feather mites interact with birds is an open challenge for evolutionary ecology.

microhabitats of the plumage where they cause no visible damage to their hosts. In fact, a few recent studies even suggest that these feather mites may be commensals or even mutualists of birds, cleaning feathers from bacteria and fungi that otherwise compromise feather structure.

On the other hand, it is shown that some species, being typical inhabitants of the downy feathers, can cause heavy bursts of mange when hosts are in unnatural or unfavorable conditions. This

briefly exemplifies how little we know about even basic aspects of feather mite biology and about the interaction between feather mites and birds.

About 2600 species of feather mites have been described in the world and many more species have yet to be discovered. Understanding the evolutionary formation and maintenance of this vast biodiversity and how these mites interact with birds are open challenges for evolutionary ecology.

“...DNA barcoding represents a leap forward that will surely stimulate and facilitate future ecological and evolutionary studies of these fascinating mites.”

A first step towards understanding the evolutionary ecology of this group is describing the network of interactions between birds and feather mite species. However, the species-level identification of feather mites is a not simple task. This is because females of some taxa and immature stages of many species are often indistinguishable by means of light microscopy. Males generally provide the best identification cues but even they require a high level of taxonomic expertise for accurate species identification.

In a recent study, we provided the largest library of DNA barcodes currently available for feather mites associated with the most abundant European passerine species and we tested the accuracy of the method. Moreover, using these tiny mites we explored other issues around barcoding relevant to their extended usability and confidence in addressing issues of molecular systematics. Through computational simulations, we also calculated the effect of sampling effort (measured as number of species and species composition) on threshold calculations. This approach provides increased confidence in calculations of thresholds, dissipating doubts about optimal library size for a particular taxonomic group. In addition, we discovered a 200-bp minibarcode region that provides the same accuracy as the full-length barcode (602 bp).

The accuracy of mite species identification was perfect but decreased when singletons (species represented by only one individual) or species of the taxonomically conflictive *Proctophyllodes pinnatus* group were included. In fact, using barcodes we confirmed previous taxonomic issues within the *P. pinnatus* group. Overall, the integrative taxonomy approach we applied made it possible to discover three new putative cryptic species as well as validate three others which were suspected to be new to science on the basis of morphological characteristics.

There is still much to be discovered about the biology of feather mites, and certainly DNA barcoding represents a leap forward that will surely stimulate and facilitate future ecological and evolutionary studies of these fascinating mites.

This study is part of the project "Quantitative barcoding of birds' feather mites: taxonomy meets ecology" (CGL2011-24466) funded by the Ministry of Economy and Competitiveness of Spain. For more information about the results discussed in this article, see DOI: [10.1111/1755-0998.12384](https://doi.org/10.1111/1755-0998.12384)

SEM of a male *Trouessartia bifurcata* (Astigmata:Trouessartiidae), collected from an Eurasian blackcap (Sylviidae: *Sylvia atricapilla*).



Image credit: Heather Proctor (University of Alberta)

H. Proctor 2015

Sixth International Barcode of Life Conference

Written by: Sarah Adamowicz, on behalf of the Conference Operating Committee, Local Support Committee and Scientific Organizing Committee

The Biodiversity Institute of Ontario and the broader University of Guelph communities are delighted to be your hosts for the upcoming 6th International Barcode of Life Conference, to be held in Guelph, Canada from August 18-21, 2015. While prior meetings have been hosted by cities spanning four continents (London 2005, Taipei 2007, Mexico City 2009, Adelaide 2011, and Kunming 2013), the 6th Conference represents a homecoming of the international barcoding community.

DNA barcoding as a formalized, standardized approach for specimen identification and species discovery was proposed in two companion articles published by Paul Hebert and colleagues in 2003. Barcoding has since expanded to an exciting international movement focused on documenting, understanding, and managing biodiversity. Yet Guelph remains the home of both the Secretariat and the largest sequencing facility for the International Barcode of Life initiative (www.ibol.org).



"...the 6th Conference represents a homecoming of the international barcoding community."

The pre-conference day (August 17th) will feature both workshops and tours of the Biodiversity Institute of Ontario. Founded in 2007, this institute is home to more than 100 staff members who curate, sequence, and analyze up to one million specimens per year. Come to see the facilities for yourself and to meet the staff—we look forward to welcoming you!

The scientific program for the conference will be hosted at several venues throughout the University of Guelph, which was founded in 1964 by the union of the Ontario Agricultural College, the Macdonald Institute, and the Ontario Veterinary College. The University has since become one of the top-rated comprehensive universities in Canada and is especially known for agricultural, veterinary, and biological science. Guelph also features a beautiful campus, especially in the summer. The campus is home to an Arboretum—located just steps from the main conference venues—featuring themed gardens as well as natural trails that showcase and preserve native Ontario vegetation.

Many of the evening programming and networking events will take delegates to the downtown core of the City of Guelph, which was founded at the junction of the Speed and Eramosa Rivers, a site that has long served as a gathering place for First Nations peoples. Likewise, the 6th Conference will serve as a coming together to share scientific advances, to exchange ideas for future projects and socio-economic applications of DNA barcoding, and, most importantly, to establish and extend relationships and collaborations. An evening program on the status of biodiversity will be held at the scenic River Run Centre downtown, and diverse socializing opportunities and dining options are available nearby.

“Southern Ontario hosts many natural and cultural highlights.”

“The 6th Conference may represent the most international of the International Barcode of Life Conferences as abstracts were received from researchers based in 55 nations.”

As August is vacation season for many, we hope you will also choose to travel to this area before the meeting or stay afterwards to enjoy our corner of the planet. Southern Ontario hosts many natural and cultural highlights. Niagara Falls is nearby, as are several national and provincial parks and the cultural treasures of Toronto. Please see the conference website for many ideas for activities for individuals, small groups, or families (<http://dnabarcodes2015.org/>).

The 6th Conference may represent the most international of the International Barcode of Life Conferences as abstracts were received from researchers based in 55 nations. The Conference Committees are working hard to ensure an exciting, stimulating, and forward-looking conference. We look forward to welcoming you!



The Arboretum at the University of Guelph with gardens and natural trails.



Above: The River Run Centre in downtown Guelph, the venue of a Special Plenary Session on the *State of Biodiversity*.

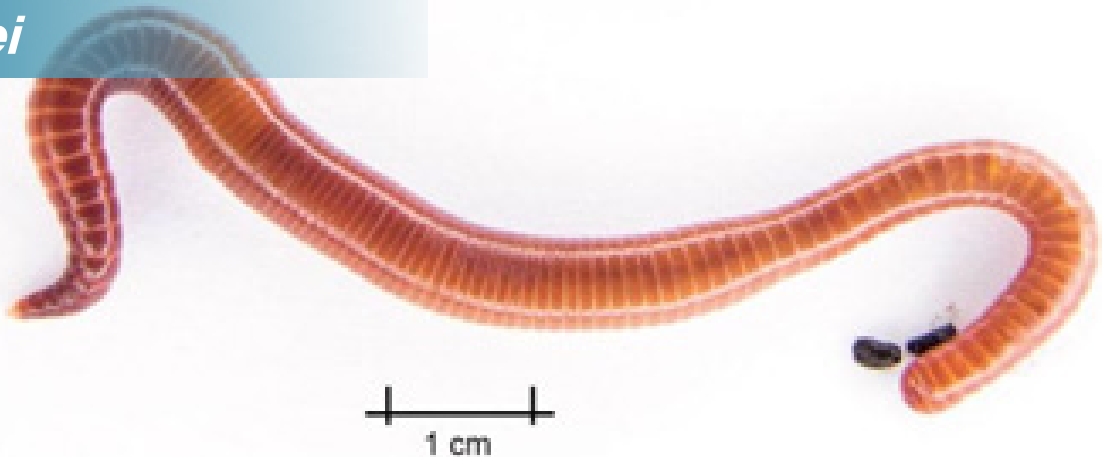
Below: The Horseshoe Falls in Niagara, located 1.5 hours from Guelph by car.



Eisenia fetida



Eisenia andrei



Barcode
Applications

Image credit: E. Roller (EOT GmbH)

Barcoding Earthworms from Ecotoxicological Test Laboratories

Written by: Jörg Römbke (ECT Oekotoxikologie GmbH, Germany), Manuel Aira, Thierry Backeljau, Karin Breugelmans, Jorge Domínguez, Elisabeth Funke, Nadin Graf, Mehrdad Hajibabaei, Marcos Pérez-Losada, Pablo G. Porto, Rüdiger M. Schmelz, Joaquín Vierna, Antón Vizcaino and Markus Pfenninger

Plenty of evidence indicates that the earthworms *Eisenia fetida* (Savigny, 1826) and *E. andrei* Bouché, 1972 (Lumbricidae) can be distinguished by morphological, physiological and molecular traits. However, the morphological differences alone do not allow

correct identification of these taxa. This may be a serious problem for the reliability of the standard ecotoxicological tests for which these worms are used.

countries on four continents. Five laboratories in Belgium, Canada, Germany, and Spain identified the specimens through DNA barcoding. All steps of the sample preparation were described by Standard Operating Procedures (SOP).

DNA barcoding is a reliable and practical method for identifying Eisenia species.

In order to assess the practicability and reliability of DNA barcoding, an international ring test was organized by the “*Eisenia* Barcoding Initiative (EBI)”, a group of scientists from four public institutions and two contract laboratories. Coded samples of *Eisenia fetida*, *E. andrei*, and *Eisenia* sp. were provided by 28 ecotoxicological laboratories from 15

The COI sequences (581 bp) obtained were used to construct a neighbor-joining tree based on the uncorrected pairwise p-distance (Figure 1). This analysis revealed three distinct haplotype clusters: one including only *E. andrei* sequences (mean within-group p - distance 0.026 ± 0.002) and two with only *E. fetida* sequences, referred to as *E. fetida* 1 and *E. fetida* 2. Each of the latter two in fact represented one single haplotype.

Only 17 out of 28 test laboratories were correct in their taxonomic assignment.

of species level differentiation. Hence, it is hypothesized that *E. fetida* 1 and *E. fetida* 2 refer to different cryptic species.

The mean p-distance between *E. fetida* 1 and *E. fetida* 2 was 0.112, whereas the mean p-distances between these two taxa and *E. andrei* were 0.142 and 0.143, respectively. Such COI divergence levels are usually indicative

As the attribution of the individual worms to these three clusters was completely consistent among the five DNA barcoding laboratories, the applicability of DNA barcoding for the identification of these ecotoxicological test species is proven. Remarkably, specimens of the molecular *E. fetida* clusters were always identified morphologically as *E. fetida*. However, this was not true the other way round, i.e. some specimens of the molecular *E. andrei* cluster were identified morphologically as *E. fetida*.

The results of this ring test were presented to standardization organizations (OECD, ISO) in order to improve the standardization and thus the quality of ecotoxicological routine testing by using DNA barcoding.

For more information about the results discussed in this article and for full affiliations of the authors, see DOI: [10.1016/j.apsoil.2015.02.010](https://doi.org/10.1016/j.apsoil.2015.02.010)

*Earthworms
used for
ecotoxicological
tests should
regularly be
(re-)identified.*

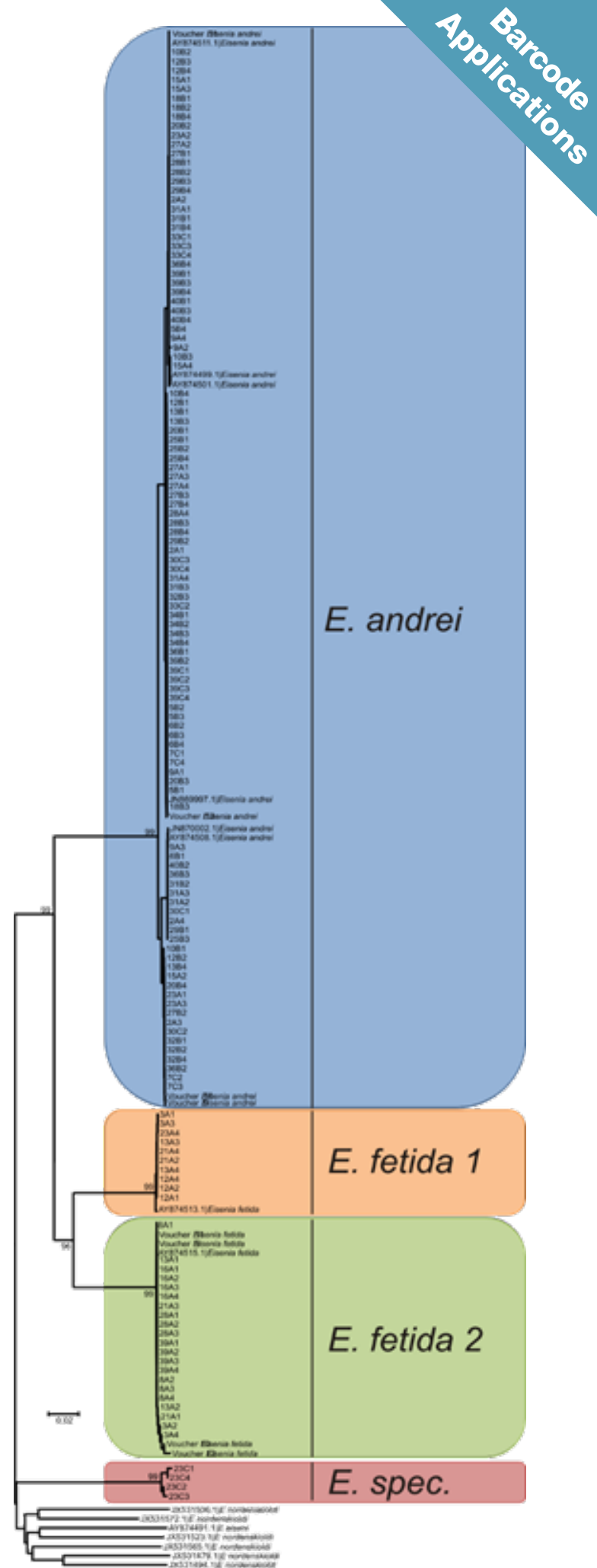


Figure 1 on right: Neighbor-joining tree of 154 test sequences together with morphologically identified voucher specimens and sequences from DDBJ/EMBL/GenBank.



Barcoding Landed Me in Prison on Robben Island

Written by: R. Greenfield and F. H. van der Bank (University of Johannesburg, South Africa)

Robben Island off the coast of South Africa is known throughout the world for its dubious past. It is where Nelson Mandela was incarcerated for 27 years and, before that, it was used as a leper colony. It has recently become a tourist attraction which is possibly contributing towards the decline of the endangered African penguin.

Little research has been conducted on the mollusks of the island with only one published paper available, which focused on one mussel species. The aim of our study was to determine the biodiversity of the more common mollusks of Robben Island, to assess if any alien

invasive species were present along the shoreline and to provide valuable data to the Barcode of Life Data Systems.

The research team stayed in the prison (converted to accommodate visiting scientists) while collecting the most abundant mollusks on the island. Samples were collected from

random sites and standard DNA barcoding protocols were used to sequence the COI region. MUSCLE (Multiple Sequence Comparison by Log-Expectation) was then used for sequence alignment. Voucher specimens were deposited at the KwaZulu-Natal Museum for species identification confirmation.

“The majority of the coastline consists of rocky shores sparsely populated with barnacles, limpets, mussels and other bivalves.”

We sampled 20 gastropod species comprising 11 genera, including three species of terrestrial snails. The most common terrestrial snail encountered was an introduced dune snail (*Theba pisana*). The Cape endemic snail (*Trigonephrus globulus*) and the European brown garden snail (*Cornu aspersum*) were also found.

The South American mussel, a bisexual, aggressive breeder that outcompetes indigenous species, was not found.

mussel species were sampled of which *Balanus glandula* and *Mytilus galloprovincialis* are exotic species. The South American mussel (*Semimytilus algosus*) was not

sampled which was particularly pleasing as it is a bisexual, aggressive breeder that outcompetes indigenous mussel species along the South African and Namibian coastlines.

The artificial substrates around the harbour were very poorly colonized, indicating that tourist ferries are not transferring alien invasive species. Abalone and mussel poaching was, however, evident.

The biodiversity data collected as part of this study are valuable and will hopefully assist managers and conservation authorities to make informed decisions on management practices on Robben Island. This is another example of how DNA barcoding can help in the conservation of unique and fragile environments.

For more information about the results discussed in this article, see DOI: [10.3897/zookeys.481.8188](https://doi.org/10.3897/zookeys.481.8188)

Scutellastra granularis scattered on the rocky shore of Robben Island.



DNA Barcoding for Research-based Learning

Written by: Joanna Werner-Fraczek (Moreno Valley College, United States)

Community colleges are not traditional research institutions. At the same time, national data indicate that an undergraduate research experience is one of the most effective paths for attracting students to and retaining them in STEM (Science, Technology, Engineering and Math) and significantly improved student performance. Implementation of research-based learning at community colleges entails overcoming certain barriers, some of which are linked to the selection of suitable research methods and techniques. DNA barcoding seems to be the perfect fit to initiate the studies at Moreno Valley College in Southern California.

With support from the Community College Undergraduate Research Initiative (CCURI), a college-wide project, Flying With Swallows (FWS), has been developed. Cliff swallows migrate to the campus from Central or South America to breed. They build multiple nests around campus, utilizing the nearby lake as a water source and the surrounding hills as a mud source for their nests.

The FWS undergraduate research project proposes two avenues of investigations. The first one, *Ecosystem Survey*, includes fauna observations on a year-round basis to monitor the presence of all birds, with an emphasis on cliff swallows, mammals, reptiles and insects on the campus. The annual fauna fluctuations are recorded on a dynamic "living" map available on the college website, constructed using ArcGIS Online and constantly updated using a smart phone application.

The second path of the FWS project focuses on *Ecosystem Investigation*, where conducted research goes beyond observations and includes biological magnification studies using swallows as monitoring organisms for pollutants. The FWS project is designed to integrate research into Biology and Chemistry courses with a common research theme.

College-wide research projects that focus on nearby ecosystems visualize STEM applications in everyday life.

The DNA barcoding technique has been employed by biology students to identify insects present on the campus and to identify swallows on a molecular level. The technique is fairly straight-forward (collect samples, isolate DNA, amplify the fragment of interest, send off for sequencing, identify the species in BOLD, supplement with morphological/phenotypic data), yet it uses database searches that bring the flavor of doing something smart and advanced.

Even more, the experiment can be easily conducted in a 30-student classroom. When students collect an insect or grow a plant, it becomes their own “pet”, therefore they immediately develop an attachment to it. Now they love what they do; biology becomes an intriguing engaging science where new technology is utilized.

DNA barcoding allows for the introduction of major molecular biology techniques to a course such as DNA extraction, PCR, electrophoresis, concentration check and database searches. With the project umbrella over the course, students develop the sense of research continuation and the individual labs are not a random collection of “who knows what for” techniques. On the other hand, the multistep procedure of DNA barcoding allows assigning different steps to non-major and major biology courses depending on the scope of the material covered. It creates a link between courses and integrates them.

Species identification using DNA barcoding supported with observational data provides material for students to design individual flyers about various species living around the campus. These leaflets, displayed on the campus hallways, promote conservation and environmental awareness. At this instant students become teachers, while DNA barcoding becomes a catalyst for a college-wide project with the number of disciplines gradually increasing.

Image credits: Daniel Pierce, Moreno Valley College



Nannostomus britskii presented the highest genetic divergence with congeners.



Comprehending the Molecular Signatures of Pencilfishes

Written by and images by: Jorge Ivan Rebelo Porto and Denise Correa Benzaquem
(Instituto Nacional de Pesquisas da Amazônia, Brazil)

The Amazon, home to more than two thousand freshwater fish species, is well known as a diversity hotspot. The identification of Amazonian fish species is challenging when using only morphology as a tool because fish taxonomists are few and the taxonomy of many Amazonian groups is still poorly known. However, species discriminations or descriptions involving cytochrome *c* oxidase subunit I (COI) appear to be a trend in Amazonian fish taxonomy mainly due to the partnership established by the Brazilian consortium for the molecular identification of biodiversity (BrBOL).

Through the innovative use of DNA barcoding, and as part of BrBOL, we analyzed the molecular signature of COI in pencilfishes. Pencilfishes are commercial ornamental fish belonging to the *Nannostomus* genus and constitute a source of revenue for the Amazonian riverine people. Despite their economic importance, little is known about their biology or life history. Pencilfishes are slender and, occasionally, some species take the unique head standing swimming posture. Since the first description of a pencilfish in 1878, a total

of 20 species have been described in the genus. Many of them have conspicuous color variations depending on their geographic location. Indeed, some of these color variants have been described as separate species in the past.

We surveyed *Nannostomus* diversity across several distant geographic locations in the Amazon Basin (Brazil, Peru, Guyana, and Suriname). Our findings recently published in the journal PLoS ONE show that the genetic narrative of pencilfishes is not the story itself but rather the telling of the story.

In 110 individuals whose DNA was sequenced, representing 14 nominal species, congeneric distance values were high. The sequence variation found defined 68 haplotypes ranging from 1–5 individuals per haplotype, without haplotype sharing between different species. Genetic distances >22% between *N. britski* and its congeners and <3% between the sister species *N. limatus* and *N. nitidus* illustrate the range of interspecific genetic variations observed in the genus.

The conspecific distance showed less than 2% divergence in 10 species. Conversely, four species presented deep lineage divergences. These divergences were evident between individuals taxonomically identified as *N. digrammus* ($9.80\% \pm 1.0\%$), *N. trifasciatus* ($8.1\% \pm 0.7\%$), *N. unifasciatus* ($7.1\% \pm 0.7\%$), and *N. eques* ($4.0\% \pm 0.4\%$). For *N. digrammus* and *N. trifasciatus*, in particular, the estimated divergences in some lineages were so high that doubt about their conspecific status is raised, suggesting the existence of hidden diversity.

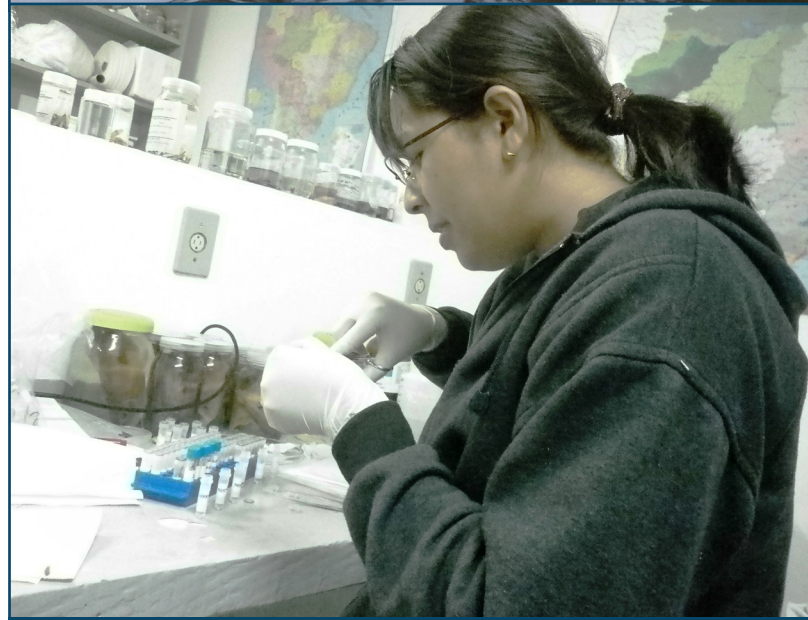
The discovery of cryptic species becomes very important when they are targets for commercial

“It was evident in this study that the cutoff values commonly used in fish do not apply to Nannostomus species...”

use. On the other hand, two species (*N. nitidus* and *N. limatus*) revealed a shallow interspecific sequence divergence (2.2%), suggesting either recent speciation or the need for synonymization. It was evident in this study that the cutoff values commonly used

in fish do not apply to *Nannostomus* species, given the high mean congeneric (19%) and conspecific (3%) distances. Pencilfishes carry their own story of evolution, written in DNA, the language of molecular genetics, but the narrative is unmistakable. Considering the economic importance of *Nannostomus* species, DNA barcoding will contribute to conservation policy in two important ways: by enhancing Amazonian biodiversity assessments to prioritize conservation areas and by providing information about evolutionary histories and phylogenetic diversity (e.g., unveiling hidden diversity). In this context, the DNA barcoding approach in pencilfishes was successfully applied, demonstrating that, rather than relying solely on morphological data, the use of molecular data should be encouraged as a supplement to *Nannostomus* systematics and taxonomy.

For more information about the results discussed in this article, see DOI: [10.1371/journal.pone.0112217](https://doi.org/10.1371/journal.pone.0112217)



Below: Representatives of the two evolutionary units observed in *Nannostomus digrammus* which presented deep genetic divergence, but no morphological differentiation.

NDIG1



NDIG2



Steppic beetles, from left: *Eusomus ovulum* (polyphagous), *Cleopomiarus graminis* (oligophagous on *Campanula* spp.), *Cryptocephalus bameuli* (polyphagous) and *Crioceris quatuordecimpunctata* (monophagous on *Asparagus officinalis*).

Image credit: M.A. Mazur and Ł. Przybyłowicz

Unravelling Ecological Interactions of Steppic Plants and Beetles

Written by: Ł. Kajtoch, D. Kubisz, W. Heise, M.A. Mazur and W. Babik (Institute of Systematics and Evolution of Animals Polish Academy of Sciences, Opole University and Jagiellonian University, Poland)

Steppic habitats sustain species-rich communities of plants and beetles, including many rare, threatened or endangered species. Steppic environments (mainly the *Festuco-Brometea* phytocoenoses) are highly threatened in Central Europe due to natural (extrazonal distribution) and anthropogenic (land transformations and patch fragmentation) factors. The steppic assemblages in Poland consist of flowering plants (over 150 characteristic species) and herbivorous beetles, mainly weevils, Curculionioidea (about 114 species), and leaf-beetles, Chrysomelidae (85 species).

Despite relatively broad knowledge about distribution and biology of steppic species, many aspects of their evolution and community ecology are poorly investigated. This may seriously limit efficiency of conservation efforts directed towards steppic habitats and communities.

During the last four years, detailed studies on beetle–host plant ecology were performed with the use of the barcoding approach; the work was supported by the Polish National Science Centre (grant UMO-2011/01/B/NZ8/01491). The

main goal of this project was to describe and understand ecological interactions between herbivorous beetles of threatened steppic environments and their host plants.

The development of a barcode library for steppic plants and beetles constituted the first, critical step of the project. To this end we collected the majority of steppic plant species known from Poland (more than 150 species) and more than half of the beetle species (59 weevils and 55 leaf beetles). The barcode library was developed for about 85% of collected plant species; this library contained three markers: *rbcl* gene, *matK* gene and *trnL* intron. Beetles were barcoded using the 5' part of the COI gene, which is widely used in beetle taxonomy and phylogenetics.

In the second step, sequences of selected plant barcodes (*rbcl* and *trnL*) were obtained from DNA extracted from beetle guts. Two sequencing technologies were applied: traditional Sanger sequencing for non-polyphagous taxa (mono- and oligophagous) and next generation sequencing with Illumina for polyphagous beetles.

By comparing sequences obtained from gut-extracted DNA to the reference barcode library, at least one host plant was identified for all of the polyphagous beetle species and for c. 67% of the non-polyphagous species. Combined analyses revealed 224 beetle-host plant interactions (117 for leaf beetles and 107 for weevils).

We found significant differences in overall diet diversity between polyphagous and non-polyphagous beetles as well as between non-polyphagous weevils and leaf beetles, but generalist weevils and leaf beetles show similar host plant utilization. Moreover, we observed that some congeneric beetles feed on the same or related plants (preferences probably inherited from common ancestors), whereas, in other genera, species feed on different plants, possibly due to adaptation to different plant chemical defenses or to reduce intrageneric competition.

For some selected species we described diet diversity on the population level, showing substantial interregional differences probably resulting from local adaptations. In addition, we demonstrated that beetles collected during harsh environmental conditions (e.g. drought or

“Combined analyses revealed 224 beetle-host plant interactions...”

cold and rainy periods) are starving which strongly reduces the efficiency of host plant barcoding.

Our results substantially extend our basic knowledge about trophic relations of beetles and plants as all previous similar studies were performed exclusively in tropical communities. This study also provides barcode libraries for steppic plants and beetles, which could be used in various applications, including biodiversity, phylogeography, conservation and ecology.

Finally, apart from the ecological implications of this study, identification of host plants for beetles could be crucial for the planning of any conservation action, including active and effective management of steppic grasslands (which needs to be rooted in basic knowledge about the ecology of both plants and associated animals) and for reintroductions or translocations of rare and endangered species of beetles.

For more information about the results discussed in this article, see DOIs: [10.1111/boj.12261](https://doi.org/10.1111/boj.12261), [10.1093/ee/nvv019](https://doi.org/10.1093/ee/nvv019), [10.3409/fb62_3.223](https://doi.org/10.3409/fb62_3.223), [10.1111/j.1744-7410.2012.00276.x](https://doi.org/10.1111/j.1744-7410.2012.00276.x), [10.1007/s10841-012-9486-z](https://doi.org/10.1007/s10841-012-9486-z)



Identifying Tarantula Endoparasitoids through DNA Barcoding

Written by: Salima Machkour-M'Rabet and Yann Hénaut (El Colegio de la Frontera Sur, Mexico)

As parasites and parasitoids are generally very small insects or arthropods, particularly as larval instars, their identification is difficult for the non-specialist taxonomist. DNA barcoding has proven that it can considerably facilitate the identification of parasitoids. Despite the fact that a large number of spiders are parasitized by a wide variety of insects, principally Hymenoptera and Diptera, some species are also parasitized by nematodes or other species of spider.

In general, there is very limited knowledge of the parasites or parasitoids of tarantula species, which is surprising considering the popularity of tarantulas as pets and their important applications in the field of medicine.

The true tarantulas only refer to species that belong to the family Theraphosidae, comprising 947 species. Mexico is characterized by a high number of tarantula species; however, only one study mentions the interaction between a *Pepsi*

species parasitoid (Hymenoptera, Pompilidae) and a teraphosid spider (*Aphonopelma* species). One widely-distributed Mexican tarantula is *Brachypelma vagans*, also known as the Mexican redrump tarantula. This particular species is desirable for the pet trade due to its attractive red abdomen and large size and is consequently protected by CITES (appendix II).

Recently, a specimen of *B. vagans* was collected in an area of southern Mexico and transported to the laboratory for reproduction purposes. Unfortunately, after several days, the spider became inactive, appeared to be uninterested in food and eventually died. The specimen was immediately placed in alcohol and after a few days numerous dipterous larvae appeared, apparently emerging from the spider. The tarantula spider exhibited a high level of parasitism, hosting 524 larvae of different sizes representing the three larval-instars, with second-instar individuals dominating.

“The tarantula spider exhibited a high level of parasitism, hosting 524 larvae...”

The identification of the endoparasitoid species was possible through DNA barcoding and the study of morphological characteristics of the larval specimens. The latter was conducted using scanning electron microscopy. We were able to identify a very common parasitoid of animal species, *Megaselia scalaris*, a cosmopolitan phorid fly. We hypothesized that *M. scalaris* adults were attracted by the accumulated remains (prey and moults) in the tarantula burrow and subsequently, the flies became parasitoids of the living spider by using the book lung as an entrance point.

These results highlight the “potential risk to the breeding of tarantulas for pets or scientific use.”

The identification of *M. scalaris* as a tarantula parasitoid is significant considering the high potential of these flies to parasitize insects or arthropods, as reported in the literature, and the potential risk to the breeding of tarantulas for pets or scientific use. The correct and appropriate implementation of tarantula breeding can, however, successfully reduce the risk of infestation. Further field research evaluating the impact of parasitoidism on natural populations of tarantulas in Mexico is also critical.

DNA barcoding was successfully applied for this research and represented an excellent alternative for the identification of *M. scalaris* larvae. In the absence of this method, morphological identification to species level is extremely difficult for non-specialist taxonomists. Given that the DNA barcoding technique allows the rapid and economic identification of species, we highly encourage its application in the laboratory by all non-specialists in molecular biology.

For more information about the results discussed in this article, see DOI: [10.1636/B14-28.1](https://doi.org/10.1636/B14-28.1)



Image credit: Humberto Bahena-Basave

Above: Larvae of *Megaselia scalaris* found in a *Brachypelma vagans* tarantula.

Below: Examples of scanning electron microscopy of *Megaselia scalaris* larvae. A - Posterior spiracular disc of a second instar larva with its two straight slits (S) for each expanded end, and the posterior spiracular hairs (PSH). B - Ventral view of the entire body of a first-instar with anterior end (AE) and posterior end (PE).

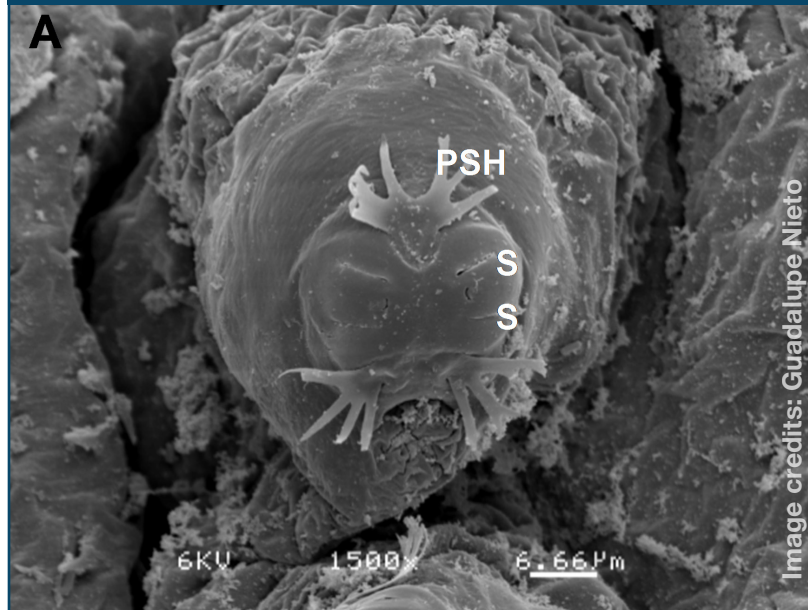
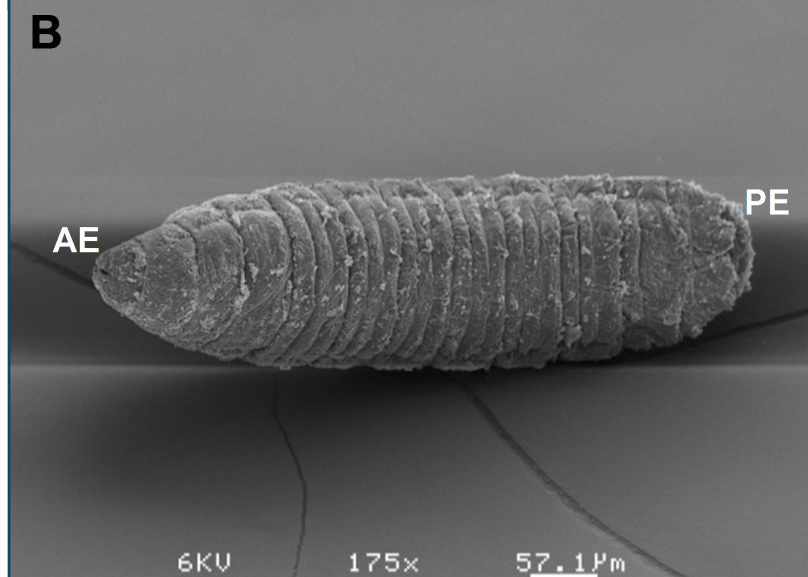


Image credits: Guadalupe Nieto



Barcoding Turkish Mosquitoes

Written by and images by: Filiz Günay (Hacettepe University, Turkey)

Turkey, at the crossroads of Europe, Asia and the Middle East, is a pathway for all living organisms. With geographical structure varying considerably over short distances, Turkey hosts a wide array of ecological habitats along with high species diversity. In addition, human movements are very dynamic among the neighboring countries including Bulgaria and Greece in the northwest and Georgia, Armenia, Iran, Iraq and Syria in the east and south. This fact makes the mosquitoes of Turkey quite important in terms of understanding the dynamics of vector-borne diseases in the area, which are closely related to socioeconomic aspects of the country.

Only two important taxonomic reviews were conducted for this interesting area. They were mostly based on research conducted during the Malaria Elimination Program which occurred between the years 1957 and 1972. A revised, annotated checklist of species was prepared in 2001 and has been our most recent source of information. Now, for the first time, we are using DNA barcoding to confirm recently collected

and morphologically identified material in connection with multiple studies.

Since 2010, we have been conducting a faunal analysis of all mosquito species in the country. Barcoding results from the genus *Culex* have proven the presence of *Cx. quinquefasciatus* and *Cx. impudicus* (instead of *Cx. territans*) for the first time and have confirmed that *Cx. pipiens f. molestus* is present and widespread.

Field trips conducted in the northwest part of the country have also revealed the presence of *Stegomyia albopicta* (*Aedes albopictus*), the famously fierce and invasive "Asian Tiger Mosquito", for the first time in Turkey. We barcoded these samples and compared them with other members of the *Stegomyia* genus, particularly to discriminate these samples from *St. cretina* (*Ae. cretinus*) because of their morphological similarities. This result has shown the necessity of initiating careful control measures focussing on this species and expanding the survey to additional areas.

In a third study, DNA barcoding provided support for eye-opening research in the field of virology. Following the detection of West Nile virus (WNV) RNA in human and equine samples in three provinces located in southern Turkey, mosquito samples were collected from 46 locations and were identified as 15 different species. Samples belonging to the species *Ochlerotatus caspius* and the *Culex pipiens* complex were found to be WNV positive, with morphological identifications being substantiated through DNA barcoding.

Further eye-opening research in the field of virology was supported with DNA barcoding.

Identifications conducted in virological studies require rapid analysis on ice in order to preserve the amount of virus in fresh samples and this can occasionally lead to misidentifications. We therefore sampled the

legs of all specimens for DNA barcoding and results showed that a *Cx. perexiguus* sample was misidentified as *Cx. pipiens* complex. In addition the rest of the samples were in fact *Cx. quinquefasciatus*, another member of this



species complex. *Cx. quinquefasciatus* is a very important vector species in the southern United States and Africa. Confirmation of its infection with WNV along with its presence in the same area as infected *Cx. perexiguus* individuals, a species known to contribute to both the enzootic cycle of the virus and transmission to equines, has allowed us to better understand the circulation of the disease in our country.

These three studies have shown the usefulness of DNA barcoding in a few aspects: clarifying the fauna and revealing new country records, detection of areas that are important for mosquito control and correcting misidentifications that occur in virological studies while preventing the loss of viruses in the samples. All of the DNA sequence data are accessible on BOLD "CXTUR *Culex* of Turkey" and "STHAW First Record of *St. albopicta* in Turkey". But this is only the beginning as we are expanding our research into all other genera of mosquitoes, especially *Anopheles*.

For more information about the results discussed in this article, see DOIs: [10.1089/vbz.2012.1093](https://doi.org/10.1089/vbz.2012.1093), [10.1016/j.actatropica.2014.10.013](https://doi.org/10.1016/j.actatropica.2014.10.013), [10.1371/journal.pntd.0003028](https://doi.org/10.1371/journal.pntd.0003028)





Image credit: Ancuta-Cristina Raclariu

Metabarcoding of European Complex Herbal Food Supplements

Written by: Ancuta-Cristina Raclariu (University of Oslo, Norway and National Institute of R&D for Biological Sciences, Romania), Mihael-Cristin Ichim (National Institute of R&D for Biological Sciences, Romania) and Hugo de Boer (University of Oslo, Norway)

The Norwegian-Romanian collaborative research project PhytoAuthent applies DNA barcoding and metabarcoding to complex herbal food supplements with the objective of evaluating the efficacy and efficiency of barcoding as a novel authentication method for these products.

Recent studies in the field of DNA barcoding of herbal supplements have highlighted widespread species substitution, adulteration and the use of unlabeled fillers. These studies have also launched a discussion about the suitability of DNA barcoding for the authentication of herbal supplements.

Both the US FDA and European EMA support the use of innovative methods but have not issued specific instructions on the use of DNA barcoding for this purpose.

In Europe less is known about the fidelity of herbal food supplement labeling, but the status quo is presumably similar to that in the United States. Monitoring and authentication of herbals in Europe is further complicated as enforcement of national and European legislation differs from country to country. The highly competitive market for these products has increased the incentive for the use of fraudulent substitutes and unlabeled fillers.

*Substitution
uncovered by
DNA barcoding is
eroding consumer
confidence in
herbals.*

Moreover, nearly unrestricted sale and access, limited medical oversight, failing enforcement of national and international regulations and insufficient monitoring of adverse drug reactions, all hamper quality monitoring of these products. Even though many have a long history of use, there are rising concerns over these products' efficacy, safety and quality in the wake of recent cases exposing discrepancies between labeling and constituents.

The project covers all major aspects concerning herbal food supplements from the legal framework to manufacturing processes and suppliers, and from consumers to safety issues and authentication. The aim is to gather information and test, develop and apply innovative molecular analysis methodologies for plant identification in herbal products, in real life case scenarios and in cooperation with local stakeholders in the herbal pharmaceutical industry.

The project investigates five cases or complexes in which substitution has varying significance: replacement of *Hypericum perforatum* by other *Hypericum* species and of *Veronica chamaedrys* by other *Veronica* species, which in both cases are likely to yield a less effective product; replacement of the threatened and protected *Gentiana lutea* by the poisonous *Veratrum album*, a substitution with potentially lethal effects; exchange substitution of *Echinacea* species depending on availability and price, which could lead to mislabeled products; and replacement of *Dactylorhiza* orchids that are both CITES-listed and protected in most countries by synthetic congeners, a case of fraudulent substitution whereby the product does not breach national and international legislation.

The herbal complexes have been carefully selected to test the efficacy of species-level discrimination using short barcodes of up to 400 base pairs on the Life Technologies Ion-Torrent PGM platform. The completed runs have used DNA extracted from complex mixtures using modified Fusion primers for nrITS1, nrITS2 and *trnH-psbA* for constituent DNA matching. At this stage we are focusing on the five cases mentioned above, but the goal is to develop high-throughput DNA barcoding for species-level identification and authentication of herbal supplements in the general case.

Funded by the Norway-Romania EEA Research Programme operated by the MECS-ANCSI PO under the EEA Financial Mechanism 2009-2014, Project Contract No. 2SEE/2014.



Image credit: Ancuta Cristina Raclariu



Image credit: Ancuta-Cristina Raclariu

Below: Herbal drug containing *Hypericum perforatum* produced by Plantavorel, one of the participating herbal pharmaceutical companies.



Image credit: PLANTAVOREL®

Funding Supports Analysis of Global Patterns in Moths

Written by: Rodolphe Rougerie (National Museum of Natural History, France)

CESAB, the Centre for Synthesis and Analysis on Biodiversity (www.cesab.org), is an initiative run by the French Foundation for Research on Biodiversity (FRB) to promote high level research activities devoted to syntheses of ideas and/or data analyses in the field of biodiversity. The CESAB program recently granted 175K€ in funding over a 3-year period to the ACTIAS project, entitled 'Global patterns of insect diversity, distribution and evolutionary distinctness - What can we learn from two of the best documented families of moths?'

The ACTIAS consortium is formed by 12 scientists from five countries (UK, Switzerland, Canada, USA and France) and will be reinforced by a postdoctoral associate and technical support. The project will include bi-annual meetings of this multidisciplinary working group at CESAB's premises located in Aix-en-Provence, in the South of France.

The ACTIAS working group will carry out a global-scale macroecology analysis for Wild Silkmoths (Saturniidae) and Hawkmoths (Sphingidae), building on the wealth of diversity, distribution, ecology and phylogeny information available for these moths, including a DNA barcode database for nearly 95% of the world species (almost 5000 in total). It will represent the first global-scale analysis of species diversity, distribution and evolutionary distinctness in a diverse group of insects. By offering important new insights into

patterns mostly documented thus far in only vertebrates and plants, the findings will serve as the basis for conservation strategies and for developing theoretical models needed to predict the impact of global changes on biodiversity.

In addition, because the spatial and temporal dynamics of speciation are expected to cause disparities in the observed patterns, we will explore the role of species traits in shaping current diversity and distributions by combining traits and comparative phylogeographic analyses in New World species. These results can provide an objective ground for fine-tuning theoretical models within subgroups and for their testing in other regions of the globe.

ACTIAS is a genus name for moths in family Saturniidae with characteristic long-tailed hindwings serving as deflectors against attacks by bats. It includes the well-known North-American Moon-Moth (*Actias luna*), but is mostly diversified in the Old-World tropics where spectacular species can be found, such as *Actias isis* or *Actias dubernardi*.



Credits and Contributions

Editors: Dirk Steinke
Emily Berzitis
Layout: 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