



BARCODE BULLETIN

The newsletter of the International Barcode of Life project

Rare lobster named after iBOL Director

A member of the iBOL Board of Directors, Jesse Ausubel, has been immortalized in the scientific name of a newly discovered, rare genus of deep water lobster. Ausubel, who is the Director of the Program for the Human Environment at Rockefeller University and vice president, programs, with the Alfred P. Sloan Foundation, was given this honour to recognize his contributions to the success of the Census of Marine Life, which he co-founded. The lobster, *Dinochelus ausubeli*, lives in the deep ocean near the Philippines.



Photo courtesy of World Registry of Marine Species

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Barcode community to convene in Adelaide

Preparations have begun in earnest for the Fourth International Barcode of Life Conference, which will be held in Adelaide, South Australia from November 26 to December 3.

Consortium for the Barcode of Life (CBOL) Executive Secretary, David Schindel, and Administrator, Stephanie Wezowicz, spent five days with the local organizing committee in Adelaide last month scouting meeting venues and local attractions. "Adelaide is a terrific place," said Schindel, "and it will be a big change for people who attended the first three barcode conferences in London, Taipei and Mexico City."

He described Adelaide as an extremely compact and fun city. "You can walk everywhere or take a free tram or bus around the downtown area. Walking around downtown you'll go from a small business district to the State Parliament, through shopping districts, past the South Australia Museum and the Art Museum, and onto the campus of the University of Adelaide which adjoins the Botanical Garden and the National Wine Centre. The Adelaide Zoo is a five-minute walk from there and everything is less than a 20-minute walk to great restaurants, including Chinatown."

Schindel says that members of the Australian barcoding community will have plenty to show their visiting colleagues. "Australia is rapidly ramping up barcoding activities. Each state has important museums, many of them participants

in barcoding initiatives like FISH-BOL and TREEBOL. A team from the Biodiversity Institute of Ontario has started a campaign to barcode the Lepidoptera in the Australian National Insect Collection. The Atlas of Living Australia will be linking barcode data to their online encyclopedia and discussions for a national network are underway."

The event will last six days with two days of pre-conference training events covering informatics and lab procedures and four days of conference sessions, including:

- Three plenary sessions;
- A session with four concurrent meetings devoted to barcoding plants, invertebrates, vertebrates and fungi/microbes;
- Two sessions with concurrent meetings devoted to barcoding taxonomic groups;
- One session with concurrent meetings devoted to multi-taxon themes (e.g., barcoding pest species, biodiversity monitoring, environmental barcoding);
- A session devoted to viewing poster presentations; and
- A half-day of free time to enjoy the attractions of Adelaide including downtown tours, excursions to the beach (20 minutes away by bus) and trips to nearby vineyards and wildlife parks. ❖

The conference website – www.dnabarcodes2011.org – is being updated regularly.

Section of DNA barcode for the rare lobster (*Dinochelus ausubeli*)



Making every species count

Barcoding in China shifts into high gear

Less than three years after the formation of China's iBOL committee, DNA barcode research in the country is moving into overdrive with several major projects in progress or ready to launch.

A major article in the *Bulletin of the Chinese Academy of Sciences* documents a period of intense activity since Scientific Director Paul Hebert led an iBOL delegation on a November 2007 visit to meet with leaders of the Ministry of Science and Technology (MOST), the National Science Foundation of China (NSFC) and the Chinese Academy of Sciences (CAS).

Under the headline **DNA Barcoding and the International Barcode of Life Project in China**, the authors – Jing Che and iBOL Scientific Steering Committee members Dawei Huang, Dezhu Li, Juncal Ma and Yaping Zhang – report that the MOU designating China as a Central Node of iBOL was signed during a visit by a high-level Chinese delegation to the Ontario Genomics Institute in April 2008.

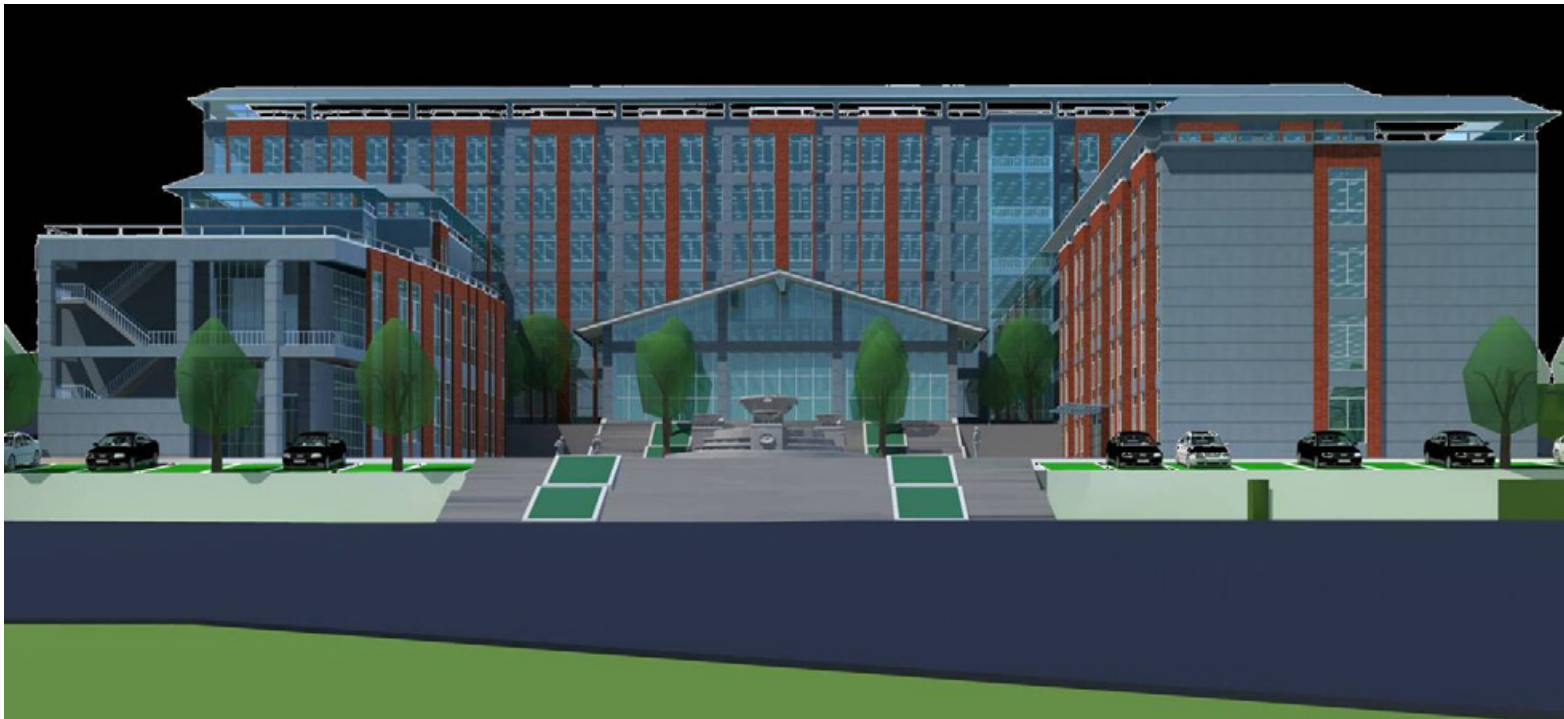
The first iBOL-China project was initiated the following September. Led by Jing Che, of the Kunming Institute of Zoology, and with 2 million yuan (US\$300,000) from the CAS Knowledge Innovation Program, the project was designed to establish the framework for iBOL-China, build and train a research team for future projects and accumulate experience in barcoding techniques and protocols.

At around the same time, MOST completed its evaluation of a major 10 million yuan (US\$1.5 million) project to establish standards and protocols for DNA barcoding, select universal primers for a variety of plant

and animal groups, construct a barcode database and obtain 50,000 barcode sequences. The project is scheduled to start this year.

Meanwhile, at a May 2009 meeting in Beijing, the seeds were being sown for a major barcoding program for Chinese plants under the leadership of the CAS-based iBOL-China committee.





Two months later, 23 specialists from 16 institutes and universities attended the first meeting of the China Plant DNA Barcoding sub-committee where a working group was formed to organize and set standards for the country's plant barcoding projects.

The first outcome was the Barcoding Chinese Plants Project, an open program associated with the Germplasm Bank of Wild Species, a major CAS facility. Led by Dezhu Li, of the Kunming Institute of Botany, the project's goals are to: Obtain

DNA barcodes systematically on a large scale and build a barcode reference library of key plant species; examine two to three standardized barcode regions that can be applied across all plants; examine variation in barcode regions for a variety of plant groups; establish the theoretical standard for designing and optimizing barcode regions; and prepare a case study for extending plant barcoding work in China.

Last year, NSFC gave the green light to a major new project to study the evolution

of DNA barcode genes and cryptic diversity. The project is led by Yaping Zhang, of Kunming Institute of Zoology, and is scheduled to start this year.

The project has multiple objectives:

- Investigate potential alternative universal barcode regions for carp, amphibians and reptiles, and fig wasps.
- Examine barcode sequence variation within and among species, especially among closely related species.
- Discover new species and cryptic species and reveal cryptic diversity in key animal groups of China and neighbouring countries.

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ABOVE: An artist's rendering of the new Kunming Institute of Biology currently under construction. When complete, it will be a core barcoding facility for China.

LEFT: Homepage of the Barcode of Life in China web portal.

- Explore the utility of large numbers of barcode sequences in revealing evolutionary processes;
- Establish the capacity for high-throughput barcode sequencing.
- Investigate new approaches to biodiversity surveys using DNA barcoding.

The CAS Bulletin also reports good progress in the construction of a separate DNA barcode data portal, storage and management system to meet China's specific needs and also a mirror site for the iBOL informatics platform, Barcode of Life Data Systems (BOLD).

China's data portal, called Barcode of Life in China, employs the international barcode data standard and includes specimen information, barcode methodology, sequence, image and associated data. The system also provides for user-based authorization and management, batch data submission, data evaluation, data search and statistical analysis. Users will also be able to compare their data to barcodes generated in other countries as well as non-redundant amino acid sequences from NCBI Genbank. Currently, it contains data sets for fish, birds, yeast and fungi. ❖



Ya-ping Zhang



The creation of iBOL-China was consolidated during a visit to the Chinese Academy of Sciences in Beijing by representatives of iBOL-Canada, the Ontario Genomics Institute and the Natural Sciences and Engineering Research Council of Canada in October 2008.

Barcode session at Pacific Science Congress

A special symposium on DNA Barcoding of Pacific Invasive and Pest Species will be a feature of the 22nd Pacific Science Congress being held in Kuala Lumpur from June 14-17. This year's congress is called Asia Pacific Science in the 21st Century: Meeting the Challenges of Global Change.

The full-day DNA barcoding symposium/workshop has three goals:

- To disseminate information on DNA barcoding and the International Barcode of Life Project;
- To provide an opportunity for local and international initiatives on the identification and control of invasive species – e.g. Quarantine Barcode of Life (QBOL) and the International Plant Protection Convention (IPPC) – to share information; and

- To identify opportunities and priorities for DNA barcoding activities on invasive species in the Pacific region.

The Pacific Science Congress is held every four years, providing a multidisciplinary platform for scientists from countries bordering the Pacific Ocean and the islands of the Pacific Basin to assess and prioritize issues requiring scientific research.

The event brings together scientists from more remote countries and catalyzes international and cross-disciplinary collaboration. It is also a venue to establish and announce new research initiatives. Recent congresses have hosted close to 2,000 participants from almost 50 countries in Asia, the Pacific islands, Australia, New Zealand, Latin America, North America and Europe. ❖

School's in for student barcoders

Efforts to get U.S. high school students engaged in DNA barcoding are gaining momentum, thanks to two new projects announced recently.

Cold Spring Harbor Laboratory's Dolan DNA Learning Center has launched the NYC Urban Barcode Project, a science competition for grade 9 to 12 students from the five boroughs of New York City.

Teams of two to four students, sponsored by an approved teacher, can use DNA barcodes to examine any aspect of the city's environment. This could include: sampling biodiversity in a park, garden,

office, or school; checking for invasive plant or animal species; monitoring animal movements or migrations; identifying exotic or endangered species in food products in markets; or detecting food or product fraud.

Project proposals will be judged for originality, creativity, relevance, plausibility and scientific merit. The teams with the best proposals will be invited to compete in the Urban Barcode Project, giving them free access to everything needed for their DNA barcode experiments, including equipment, protocols, and reagents for DNA extraction, amplification, and DNA sequencing.

Teams must complete their projects by next spring and present their work at a project symposium. The best overall project will win US\$10,000, with an extra \$10,000 in runner-up prizes.

The Urban Barcode Project, funded by the Alfred P. Sloan Foundation, will provide infrastructure to create "citizen scientists" by involving a large number of students in barcoding experiments producing data that is potentially useful to for various applications. For more information:

www.urbanbarcodeproject.org/


Meanwhile, on the west coast, Coastal Marine Biolabs (CMB) has announced that it is joining forces with Bio-Rad Laboratories Inc. to develop DNA barcoding instructional activities for the classroom. In a press release, CMB said that the collaboration merges Bio-Rad's expertise in the development of instructional tools offered through its Biotechnology Explorer program with CMB's expertise in engaging students in the generation and submission of real DNA barcode data to iBOL. The result of this collaboration between the two California-based companies equip educators with innovative tools to promote bioliteracy and increase their students' interest in scientific discovery. ❖



Going Up!

March 1 was a construction milestone for the new Centre for Biodiversity Genomics at the University of Guelph as the laying of the floor for the first-story marked the completion of major work on the below-ground section. The C\$18 million research facility is scheduled for completion in January 2012. ❖





Taking it outside: ACG fieldwork reveals more about cryptic species

By Claudia Bertrand*

In the course of developing their international reputation as pioneers in tropical conservation, Dan Janzen and Winnie Hallwachs have pursued a long-term, complex inventory of Lepidoptera, their host plants and parasitoids in Costa Rica's Area de Conservación Guanacaste (ACG). This basic ecological study includes the most extensive larval and parasite rearing experiment and wild adult collections anywhere.

DNA barcodes are integral to this vast and growing inventory. ACG's collaboration with International Barcode of Life founder Paul Hebert dates to barcoding's inception in 2003. Tiny Costa Rica is the largest non-Canadian contributor nation to the Barcode of Life Data Systems (BOLD) repository, largely

on the basis of the work being done in ACG with contributions from INBIO, the country's national biodiversity institute.

In considering my Masters research plan at the Biodiversity Institute of Ontario (BIO), the large barcode sets of Janzen and Hallwachs' ACG inventory, recorded in BOLD, offered a rich source of data for analysis at the margins of species distinction and identification. To date, DNA barcodes have reliably identified morphologically defined species of moths, butterflies and parasitoid flies and wasps with 99 percent accuracy when compared to the existing barcode library. The addition of DNA barcodes to the Costa Rican ACG inventory has also shed light on morphologically cryptic species complexes.

Currently, 12 percent of previously morphologically-defined ACG Lepidoptera species separate into two or three clusters of barcodes when visualized on a neighbour-joining (NJ) tree. These "flagged" species complexes range in divergence levels from very shallow (less than 1 percent) to as deep as 8 percent in their 650bp COI sequence. Scrutiny by experienced taxonomists revealed many cryptic species of Lepidoptera, such as the famous *Astrapes fulgerator*. About half of these cases had associated morphological and/or micro-ecogeographical correlates.

However, experienced (or still active) taxonomists with an understanding of certain Lepidoptera families are increasingly hard to find. This shortage in alpha taxonomists, coincident with advancements in molecular techniques, has inspired me and other researchers to study molecular mechanisms that explain what is actually happening in these potentially cryptic species complexes.

*Claudia Bertrand, a second-year M. Sc. student in the lab of Prof. Mehrdad Hajibabaei at the Biodiversity Institute of Ontario, has just returned from Costa Rica's Area Conservación Guanacaste where she investigated the barcoding of cryptic species complexes in the field, testing morphology, taxonomy and ecology against NJ (neighbour joining) clusters.



Above: ACG parataxonomist Johan Vargas helps Claudia to collect insects from a malaise trap. (Photo by Brad Zlotnick)



Left: Tropical biology pioneers Dan Janzen and Winnie Hallwachs in the field.

About ACG

Area de Conservación Guanacaste (ACG), a 160,000-hectare UNESCO World Heritage Reserve in northwestern Costa Rica, incorporates national parks and other lands managed for biodiversity conservation and survival through wildlife, forest and agricultural research in its primary and secondary dry, rain and cloud forest, and marine ecosystems.

The conservation area covers two per cent of the country and contains an estimated 350,000 species, representing about 65 percent of the species in Costa Rica, or as much as the entire USA.

The ACG is a dynamic biocultural resource for Costa Rica, providing education and employment for the local people who staff the area, run research and tourism programs, fight fires and sustain groundbreaking field research as parataxonomists. University of Pennsylvania biologists Dan Janzen and Winnie Hallwachs have been technical advisors to this world renowned research venue for more than 25 years. It is here that they conceived and implemented many of the innovative tropical biology concepts that have made them famous.

The tropics, especially Central America, are known to harbour overlaid cycles of colonization from newly connected land masses, as well as deep genetic divergences over short geographical distances. As a result, distinct lineages can be confined to small areas, seemingly minor ecological circumstances and narrow elevation zones. All this adds to the complexity and importance of cataloging hidden diversity in tropical settings.

Ribosomal gene fragments and many other alternative secondary genetic markers are now being tested. Population level analyses are also identifying reproductive isolation in apparent cryptic speciation within the ACG. Expanded approaches to analyzing cryptic species will further elucidate the strengths of DNA barcoding, help us more accurately catalog biodiversity, and assist conservation management decisions in the tropics.

My fieldwork in ACG has verified findings in the BIO labs and given new direction to my thesis work. Molecular genetic approaches will augment current reproductive lineage experiments in the ACG as we learn more about cryptic species complexes and their

implications for biodiversity science. I am focusing on cases that appear to be one species morphologically, behaviorally and ecologically, but that show one of two kinds of split.

Most puzzling, is when many tens of individuals bear one or two almost identical barcodes, but one individual differs in as many as 5-10 base pairs. The other, hopefully more tractable, case is where what seems to be one species divides into two sympatric barcode lumps of approximately the same size but 1-8 percent different in their barcodes.

Forest encounters with the diverse Lepidopteran and parasite life stages, and the field conditions and parataxonomists who find and rear them, gave me a deeper understanding of the ecological milieu for our ongoing lab genetics. Going forward, my work will use the various molecular approaches discussed above to move toward the next round of questions generated by my trip, such as elevating cryptic species to “nameable” species status and understanding hybrid zones in short geographical regions.✿

Barcoders welcome new rules for shipping specimens

The DNA barcoding community is welcoming the news that natural history specimens shipped via commercial airlines will no longer be classified as “dangerous goods.”

In a press release, the Natural Science Collections Alliance said that the new policy, issued by the International Air Transport Association (IATA), also removes a requirement for shippers to be formally trained



in how to package scientific specimens. Instead the shippers can train themselves.

“This is a major change in the requirements for shipping biological specimens in alcohol, and will reduce the time and expense of such shipments considerably,” said Scott Miller, Deputy Under Secretary for Collections and Interdisciplinary Support at the Smithsonian Institution and vice-chair of iBOL Working Group 2.2 – Museum Life.

“Thus, it is a major step forward for facilitating the movement of specimens for barcoding and taxonomic research, both within countries and between countries, and will be very helpful in building iBOL collaborations.”

These special provisions, listed as A180

in the 52nd edition of the IATA Dangerous Goods Manual, came into effect on January 1. The new regulations will enable researchers and collections curators to ship specimens more easily and to expand the list of countries to which these materials can be sent.

The U.S. Postal Service will also exempt the shipment of natural history specimens from its list of dangerous goods shipped domestically. To qualify for the exemption, specimens must be placed in three layers of heat-sealed bags and contain no more than 30ml of free liquid. Scientific specimens will still not be allowed in carry-on or checked luggage.

The new regulations can be downloaded at <http://tinyurl.com/4v1kwzm> ✿

Oil changers: researchers use barcodes to sniff out phony olive oil

Is that expensive extra virgin olive oil you bought the real deal, a mixture of olive oil and some other cheap vegetable oils, or an entirely olive-free zone? DNA barcoding is an accurate and rapid way to find out, according to new research published recently.

With high demand and high prices for extra virgin olive oil, adulterated olive oil has become the biggest agricultural fraud problem in the European Union, according to the International Olive Oil Council, an intergovernmental organization based in Madrid. With a booming export market for the top-grade oil, it is a problem that affects virtually every region of the world.

The substitutions range from oil labeled as “extra virgin” diluted with cheaper olive oils or other vegetable oils to many cases

where “extra virgin olive oil” is actually low-grade soy or canola oil coloured with industrial chlorophyll and flavoured with beta-carotene.

Now researchers are reporting the results of a study in which the objective was to establish the authenticity of olive oil based on species-specific DNA markers.

The research team – Shashi Kumar of the International Centre for Genetic Engineering and Biotechnology, New Delhi; Talwinder Kahlon, of the US Department of Agriculture, Albany, California; and Sarika Chaudhary, of the University of California, San Francisco – report on their findings in the journal *Food Chemistry* under the heading “A rapid screening for adulterants in olive oil using DNA barcodes”.

“The PCR amplified DNA sequences from contaminated olive oil were matched with consensus DNA sequences generated for canola and sunflower oil to unmask the cross-contamination,” the research team reported.

They concluded that “the combined approach of molecular biology and bioinformatics technology can be used as an inexpensive method for ensuring the purity of olive oil”. ✿

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