

# DNA Barcoding, Capacity Building, and CPM's Mission

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### Molecular Identification of *Ceratitis capitata* (Tephritidae) and related fruit flies: Transitioning into the DNA Barcode Era

Norman B. Barr<sup>1</sup>, M. Saidul Islam<sup>1</sup>, Bruce A. McPherson<sup>1</sup> & Peter De Meillon<sup>2</sup>

**Objective**  
The development of a DNA barcode for the identification of agricultural pest species of the genus *Ceratitis* is a key step in the transition to the DNA barcode era. This poster describes the development of a DNA barcode for *C. capitata* and its application in the identification of pest species.

**Tephritid Barcoding Initiative**  
In April 2008, the Consortium for the Barcode of Life (CBOL) initiated the Tephritid Barcoding Initiative (TBI) to develop a DNA barcode for the identification of pest species of the genus *Ceratitis*.

**Introduction**  
The genus *Ceratitis* comprises several species of agricultural pest species. The identification of pest species is a key step in the transition to the DNA barcode era. This poster describes the development of a DNA barcode for *C. capitata* and its application in the identification of pest species.

**Materials and Methods**  
A total of 100 individuals were collected from various locations in South Africa. The DNA was extracted and the COI gene was amplified using PCR. The resulting PCR products were sequenced and the resulting sequences were aligned and compared to those of other *Ceratitis* species.

**Results and Discussion**  
The results of the sequencing and comparison of the COI gene sequences are presented. The results show that the COI gene sequences of *C. capitata* are highly similar to those of other *Ceratitis* species, but distinct enough to allow for identification.

**Conclusions**  
The results of this study demonstrate that the COI gene is a suitable marker for the identification of pest species of the genus *Ceratitis*.

**References**  
Barr, N. B., Islam, M. S., McPherson, B. A., & De Meillon, P. (2008). Molecular identification of *Ceratitis capitata* (Tephritidae) and related fruit flies: Transitioning into the DNA Barcode Era. *Journal of Applied Entomology*, 142(1), 1-10.

### Turning DNA barcodes into an alternative tool for identification: African fruit flies as a model

Elisa M. S.

Pharmaceuticals/Plant Systematics/Plant Systematics  
Norman Barr<sup>1</sup>, Bruce A. McPherson<sup>1</sup>, Peter De Meillon<sup>2</sup>, Norman B. Barr<sup>1</sup>, Saidul Islam<sup>1</sup>, Bruce A. McPherson<sup>1</sup> & Peter De Meillon<sup>2</sup>

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**Advantages of DNA Barcoding**  
DNA barcoding is a simple and cost-effective method for the identification of pest species. It is also a highly accurate method for the identification of pest species.

**Acknowledgements**  
This work was supported by the National Research Foundation of South Africa.

### QBOL - Identification of phytoplasmas using DNA 'barcodes'

Nicoletta Contador<sup>1</sup>, Olga Makarova<sup>1</sup>, Sanna Pahlsson<sup>1</sup>, Assunta Bertozzi<sup>2</sup>, Margareta Nicolaisen<sup>3</sup>

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**Objective**  
To identify phytoplasmas using DNA 'barcodes'.

**Introduction**  
Phytoplasmas are plant pathogens that cause a wide range of diseases in agricultural crops. The identification of phytoplasmas is a key step in the management of these diseases. This poster describes the development of a DNA barcode for the identification of phytoplasmas.

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### QBOL: Developing DNA barcode identification for Q-organisms

QBOL Barcoding of Life

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# DNA barcoding and forest biosecurity

L.M. Humble, J.R. deWaard, R. Hanner and P.D.N. Hebert

## Introduction

The ability to distinguish non-indigenous species (NIS) from native species is critical to the success of any surveillance program. Unfortunately there are numerous problems inherent with detection of NIS including:

- large samples needed to detect NIS when they are present at low levels
- immature life stages often intercepted but usually cannot be fully identified
- often inadequate knowledge of native fauna

We provide examples from forest bio-surveillance in Canada showing how these problems can be circumvented by the application of DNA barcoding (DNAB) for species identification.

## What is DNA barcoding?

- DNAB uses the sequence variation in a short, standardized DNA fragment to identify organisms
- DNA fragment used for animals is a 658 base pair segment of the mitochondrial gene cytochrome c oxidase subunit I (COI) (Hebert et al. 2003)
- It compares unknown sequences against a reference library of DNA sequences
- It meets or exceeds minimum standards required for diagnostic protocols under ISPM No. 27 (Floyd et al. 2010)



Figure 1. Light trapping for nocturnal moths.

## Biosurveillance for NIS

### Methods

- moths sampled with UV lights (Fig. 1)
- a single leg was removed and barcoded using standardized procedures (Fig. 2) (deWaard et al. 2009)

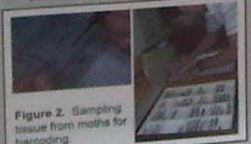


Figure 2. Sampling tissue from moths for barcoding.

## Biosurveillance (cont'd)

### Results

The identification engine in the Barcode of Life database (BOLD-ID) was used to obtain initial identifications for the 925 specimens (Fig. 3).

- ~190 species clusters with a 3% sequence divergence cut-off (Hebert et al. 2003)
- 124 clusters assigned to species, 61 to genus using BOLD-ID (all species assignments were also confirmed morphologically)
- 66 remaining clusters identified morphologically (the only step in the identification process that required taxonomic specialists)



Figure 3. Species identification report and neighbour-joining trees from BOLD-ID.

- 31 species and 16% of all moths captured were NIS
- two NIS, *Argyresthia pruniella* and *Dicheilonia histriovana* (Fig. 4), were new introduction records for North America
- two NIS, *Parasitomeria latorea* and *Prays fraxinella* recorded for the first time from western Canada

DNA barcoding provides an efficient and rapid means of assessing large samples. It enhances both species recognition and the detection of new NIS by:

- minimizing valuable specialist time;
- detecting species at low density

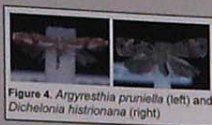


Figure 4. *Argyresthia pruniella* (left) and *Dicheilonia histriovana* (right)

## Identification of immature life stages

Eggs, larvae or pupae are the most frequently intercepted life stages of many quarantine organisms. They usually cannot be fully identified to species.

- DNA barcodes are invariant during a species development; any life stage can be identified from its DNA sequence
- European poplar shoot borer, *Gymnosoma sicrana*, was first reported from North America in 2001

- barcoding of adults in museum collections confirmed its long-term presence in western Canada (Fig. 5)
- barcoding of larvae from delimitation surveys used to define its range in British Columbia (Humble et al. 2009)



Figure 5. Neighbour-joining tree of COI sequences and geographic origin of samples for *Gymnosoma sicrana*. Larval samples are denoted with italics.

## Building DNA reference libraries

DNA barcoding identifies unknown species by comparing their COI sequences to reference sequences derived from reliably identified species sampled from museum collections. Development of the sequence libraries is done in collaboration with taxonomic specialists. Two examples of reference library development follow.

# DNA barcoding and forest biosecurity (cont'd)

## Building DNA reference libraries

### 1. *Lymantria* tussock moths

*Lymantria* includes serious quarantine & forest pests (e.g. gypsy moth (*L. dispar*), pink gypsy moth (*L. matshura*) and nun moth (*L. monacha*)).

- Species are often transported globally as dormant egg masses on vessels and cargo
- DNA reference library constructed for the identification of 36 *Lymantria* spp. (deWaard et al. 2010a)

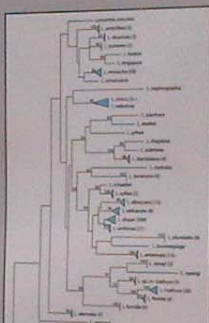


Figure 6. Maximum likelihood tree for 36 species of *Lymantria* constructed with the barcode region of the COI gene. The number of specimens sampled per species is noted in parentheses (after deWaard 2010).

### Results

- 518 adult *Lymantria* from 35 countries barcoded (Fig. 6)
- barcode data led to morphological and taxonomic re-evaluation of specimens in two clusters (deWaard et al. 2010a)
- *L. sp. nr. matshura* (Fig. 6) now considered to be *L. subpallida*
- *L. nebulosa* is a valid species distinct from *L. sinica*

## *Lymantria* spp. results (cont'd)

- 142 COI haplotypes identified across all 36 species
- no haplotypes shared between species
- 91 COI haplotypes within *L. dispar*
- haplotypes of Asian subspecies *L. d. asiatica* and *L. d. japonica* cluster separately from European & North American subspecies *L. d. dispar*
- allows rapid identification of "Asian gypsy moth" recovered from monitoring programs

## Geometridae

DNA reference library was developed for the 349 spp. of Geometridae in British Columbia (deWaard 2010).

- specimens from 8 museum collections sampled, databased and imaged, DNA extracted and COI sequenced
- all data is publicly available from the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007) in project 'GOBCL - Geometridae of BC Library'

### Results

- 2392 COI sequences generated from 400 species in 125 genera
- 374 (93.2%) of the species could reliably be distinguished with barcodes
- only 27 species (6.8%) had undifferentiated or overlapping barcodes
- both a new NIS (Fig. 7) and a new native species for Canada were detected by barcoding museum collections (deWaard et al. 2008, 2010b)

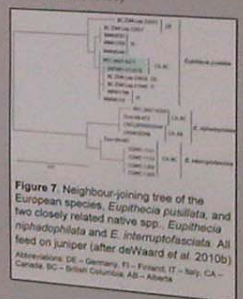


Figure 7. Neighbour-joining tree of the European species, *Eupithecia pusillata*, and two closely related native spp., *Eupithecia niphadophila* and *E. eupithecia*. All feed on juniper (after deWaard et al. 2010b).

Abbreviations: DE - Germany; IT - Finland; IT - Italy; CA - Canada; BC - British Columbia; AB - Alberta.

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deWaard, J.R., Schmidt, B.C.S. and Humble, L.M. 2010b. DNA barcoding flags the first North American record of a Eurasian moth, *Eupithecia pusillata* (D. & Schiff, 1775) (Lepidoptera: Geometridae). *J. Entomol. Soc. Brit. Columbia* 107: 1-4.

Floyd, R., Lima, J., deWaard, J., Humble, L. and Hanner, R. 2010. Common goals, policy implications of DNA barcoding as a protocol for identification of arthropod pests. *Biological Invasions* 12 (9): 2047-2054.

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**For further information**

Please contact the authors (see below) for publications. More information on these and related projects can be obtained from the Canadian Centre for DNA Barcoding - <http://www.cccb.ca> or the Barcode of Life Data Systems - <http://www.biodidates.org/view/login.php>.

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Industry & Investment



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# INSECT PEST DIAGNOSTICS & SPECIES DISCOVERY UNDER IBOL: THE CASE OF OROSIUS LEAFHOPPERS

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## Introduction

*Orosius* Distant (Hemiptera: Cicadellidae) is a leafhopper genus of economic importance in Africa, the Middle East, Asia, Australia and the Pacific. At least half of the eight described species have been reported as vectors of plant pathogens, including phytoplasmas and viruses. Despite their economic importance, the identities and distributions of *Orosius* species are unclear and the genus has a checkered taxonomic history. In addition, like many leafhoppers, only adult males of *Orosius* are identifiable to species. The resulting uncertainty over the identity of vector species has hampered research into phytoplasma transmission and management.

We performed an integrative taxonomic study of the genus in order to provide tools for elucidation of the vector capability of *Orosius* species. DNA barcodes available for seven of the eight recognised species and several putative novel species were used to form a species clusters using a general mixed Yule-coalescent (GMYC) modeling procedure (1). These clusters were compared with morphospecies clusters identified through male genitalia characters.

## Results & Discussion

Multiple threshold GMYC modeling applied to 97 unique *Orosius* haplotypes identified 18 genetic clusters (Fig. 1). These clusters resolved as seven previously described morphospecies, three novel morphospecies and five novel species represented by females only (Fig. 2). Average sequence difference within morphospecies was 14 times less than that observed between morphospecies (0.76% vs 11.4%). Monophyly at each morphospecies was well supported by bootstrap analysis. There was strong congruence between species clusters defined by genetic and morphological methods. DNA barcoding was very useful for delimiting morphologically cryptic species where male genitalia character variations were subtle (eg. *O. argentatus* vs. *O. orientalis*). Use of ♀ samples in genetic analyses allowed detection of additional putative novel species (Fig. 2).

## Conclusions

Our study doubles the known species diversity of the genus and clarifies the identity and distributions of the species. The implications of newly discovered cryptic species for studies of plant disease transmission are significant. Our new diagnostic tools can be used as a standard for future investigations of leafhopper-phytoplasma species associations.

**References**  
1. Zhang, J., Shi, J., Wang, J., & Li, D. (2006). A general mixed Yule-coalescent model for species delimitation. *Molecular Biology and Evolution*, 23(12), 2691-2701.

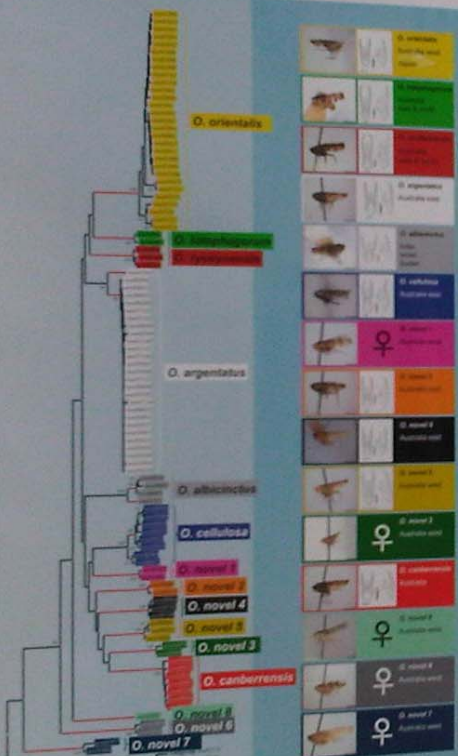


Figure 1. NJ tree of 97 unique *Orosius* haplotypes derived from 179 specimens. Red branches indicate genetic species identified by multiple threshold GMYC model.

Figure 2. *Orosius* morphospecies, male genitalia characters and sample distributions. ♀ = female specimens only; putative novel species discovered exclusively by genetic.



# THE INTERNATIONAL BARCODE OF LIFE PROJECT

## Bringing Genomics to the Battle Against Plant Pests and Invasive Species

The International Barcode of Life Project is building the global infrastructure for rapid, accurate and inexpensive species recognition using a DNA-based identification system. DNA barcoding is a new technology with immense potential for economically, socially and environmentally beneficial applications – none more important than agricultural and forestry pest management, identification of invasive species and customs-border protection. In insect pest outbreaks, the rapid identification of the organism is critical for the implementation of control strategies to minimize lost production. The ability to identify parasitoid species that are either important in controlling outbreaks or in biological control is also crucial.

## IBOL WORKING GROUP 1.5

### Agricultural and Forestry Pests and their Parasitoids

Members associated with IBOL Working Group 1.5 are assembling a DNA barcode repository of agricultural and forestry pests and their parasitoids by 2015. This digital reference library will contain barcodes for at least 25,000 of the most economically important pest species from every region of the world. Despite the broad range of groups involved, a collection of digital records for 25,000 species will provide a comprehensive and extremely effective pest-control identification system.

Group	Species	IBOL-GBOL Project	Species	Progress	
Tree Bark	5,000	712	14%	2,000	50%
Europe	100	18	18%	20	20%
Greenhouse	500	151	30%	500	100%
Lawrence & Co	500	129	26%	277	55%
Mexico	100	9	9%	155	155%
Parasitic Flea	3,500	550	16%	1,820	61%
Parasitic Hymenoptera	8,000	2,245	28%	8,351	79%
Plant Flea	2,000	874	44%	1,800	90%
Phytophagous Beetles	4,000	1,616	38%	3,700	94%
Flies	700	80	11%	244	41%
Black Flies	700	23	3%	28	4%
Thrips	1,000	96	10%	200	20%
<b>Global Total</b>	<b>25,000</b>	<b>8,654</b>	<b>35%</b>	<b>17,750</b>	<b>71%</b>



## AN IBOL HIGHLIGHT

September 25, 2010  
The Global Assembly of the International Barcode of Life Project was concluded at the University of Queensland, Australia. To mark the occasion, the event was dedicated to the 19th International Conference on Systematics, the 10th International Congress of Systematics, and the 10th International Congress of Entomology.

## IBOL IN KENYA

Kenya is a world leading in biodiversity reference collection of the country's insects. Among the hundreds of species recorded in Kenya, many are new to science, including the first Kenyan species of *Orosius*. To mark the occasion, the event was dedicated to the 19th International Conference on Systematics, the 10th International Congress of Systematics, and the 10th International Congress of Entomology.

## IBOL IN MEXICO

Numerous genetic diversity hotspots of agricultural pests and invasive species are underway in Mexico. IBOL Working Group 1.5 is currently working to identify and catalogue the genetic diversity of these species. This work will provide valuable information for pest management and conservation efforts. IBOL Working Group 1.5 is currently working to identify and catalogue the genetic diversity of these species. This work will provide valuable information for pest management and conservation efforts.



## A RAPID AND RELIABLE DIAGNOSTIC PROTOCOL



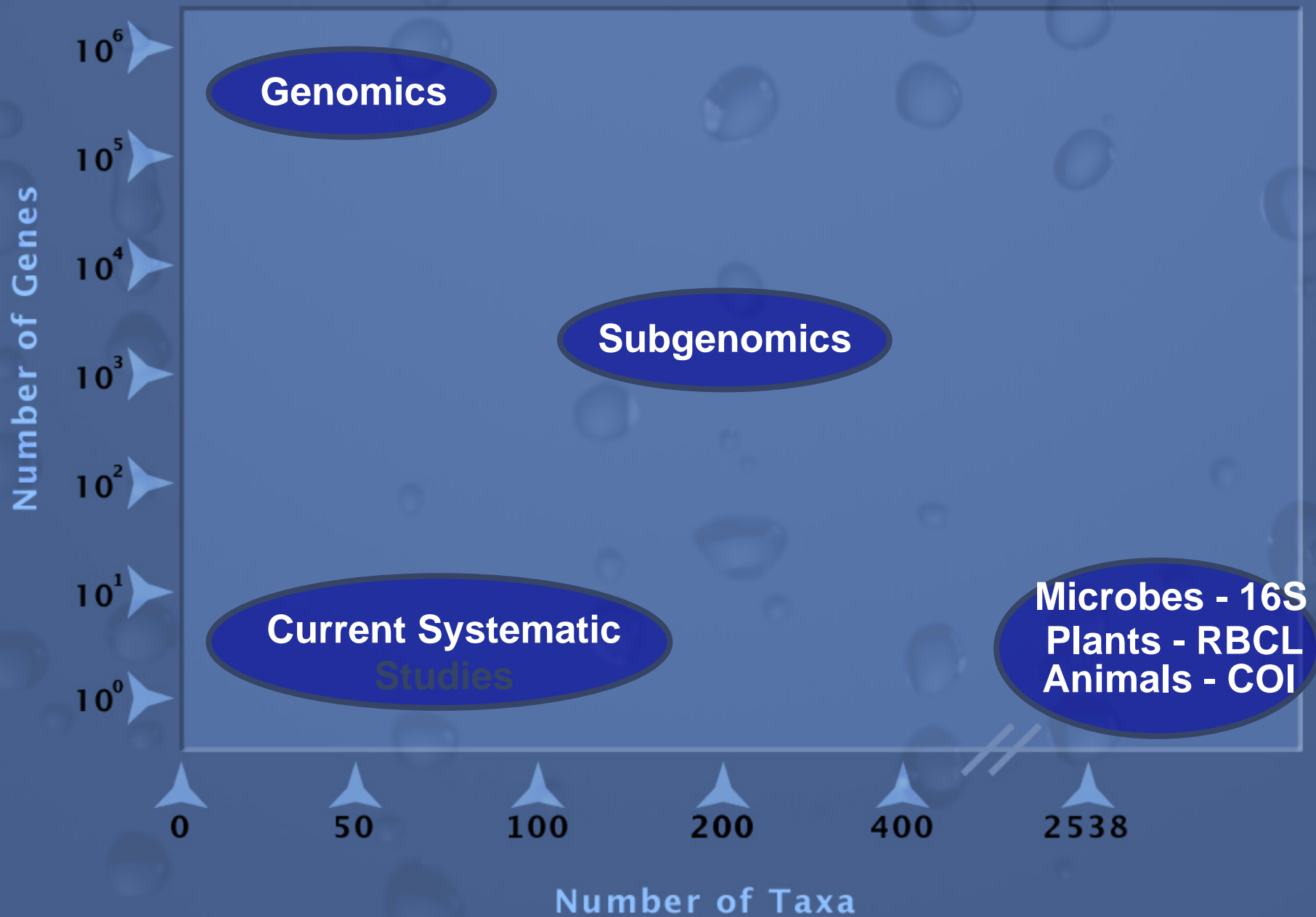
In 2009, DNA barcoding was used to identify a pest species. A DNA barcode was generated from the pest's DNA. This barcode was then compared to a library of DNA barcodes for known species. The result was a match to a specific species, allowing for rapid and reliable identification. This process is being used to identify and catalogue the genetic diversity of agricultural pests and invasive species in Mexico. IBOL Working Group 1.5 is currently working to identify and catalogue the genetic diversity of these species. This work will provide valuable information for pest management and conservation efforts.

# Species Identification Matters to All Countries

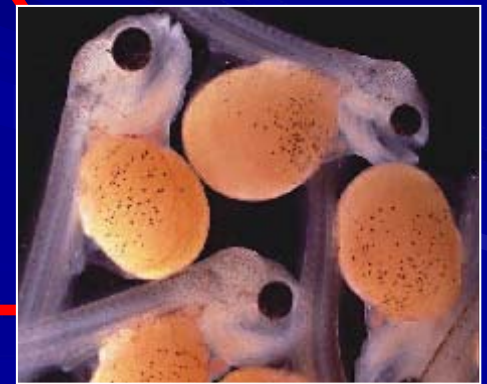
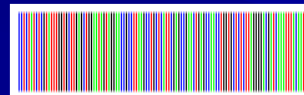
- Food security and safety
- Border inspection and trade agreements:
  - Agricultural pests/beneficial species
  - Disease vectors/pathogens
  - Endangered/protected species
  - Invasive species
- Ensuring ecosystem services
- Environmental quality assessment
- Documenting/developing genetic resources
- University research in biology



**A DNA barcode is a short gene sequence taken from standardized portions of the genome, used to identify species**

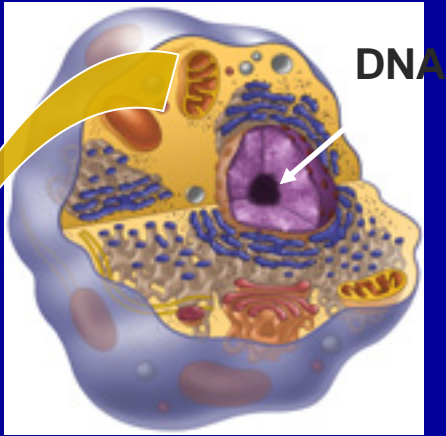


# Associating Life Stages, Processed Parts, Dimorphic Genders

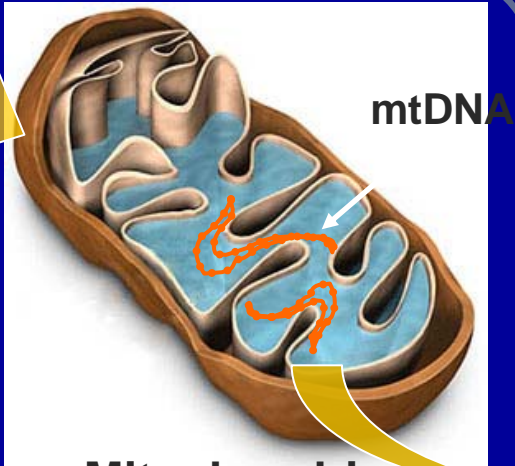




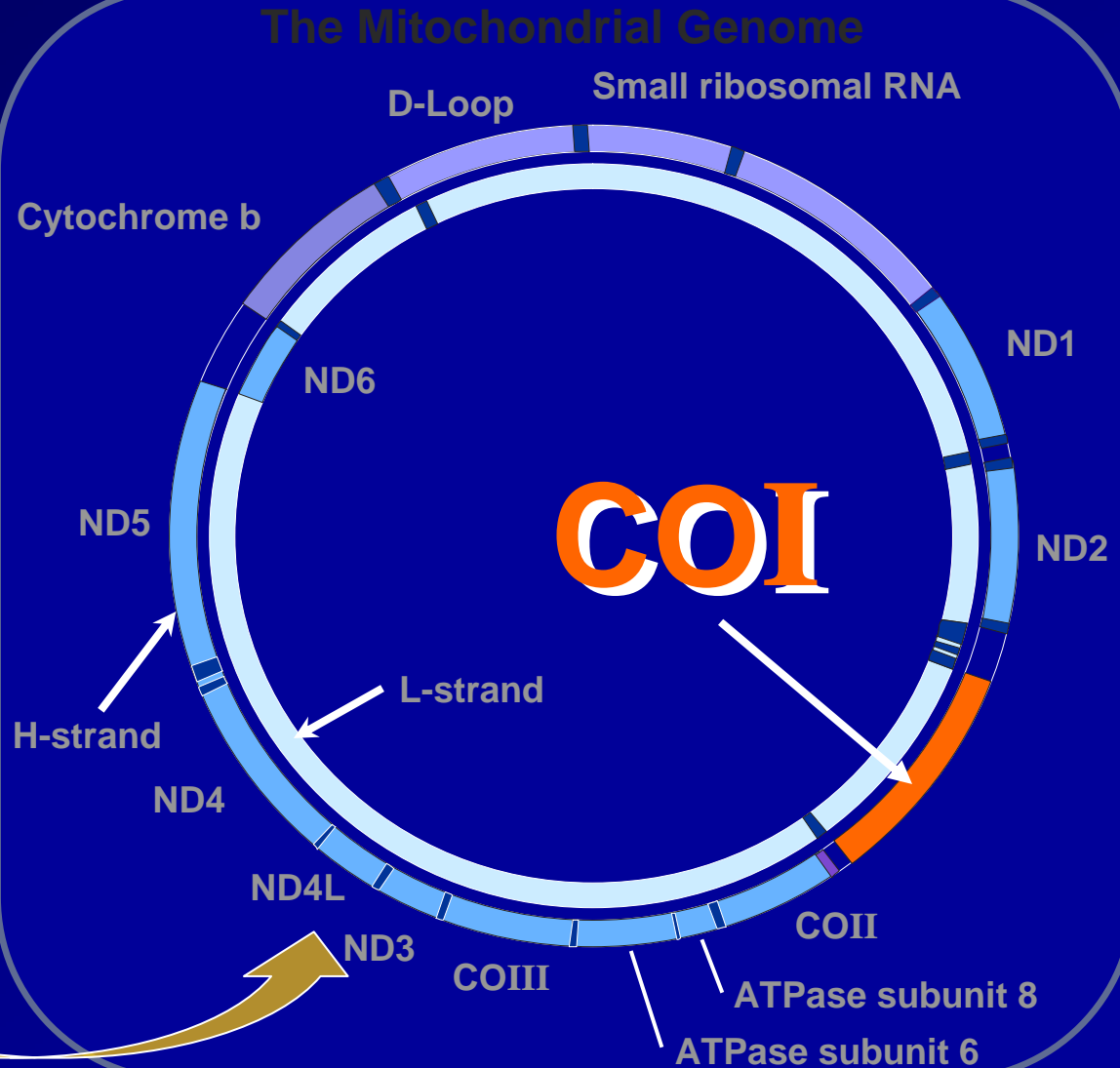
# An Internal ID System for All Animals



Typical Animal Cell



Mitochondrion



# Non-COI regions for other taxa

## ■ Land plants:

- Chloroplast *matK* and *rbcL* approved Nov 09
- Non-coding plastid and nuclear regions being explored

## ■ Fungi and protists:

- CBOL Working Groups convened
- Recommendations expected in 2010

Biol Invasions

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PERSPECTIVES AND PARADIGMS

## **Common goals: policy implications of DNA barcoding as a protocol for identification of arthropod pests**

**Robin Floyd · João Lima · Jeremy deWaard ·  
Leland Humble · Robert Hanner**

**“DNA barcoding already meets or exceeds the minimum standards required for diagnostic protocols under ISPM No. 27.”**

# Barcoding in Diagnostic Protocols

- Applicable to all life stages
- Usable by non-experts
- Well-documented standard lab procedures
- High degree of transparency and repeatability
- Protocols, results, documentation all public and archived
- IDs and specimen comparisons through digital data, objective criteria
- Linkage to reference voucher specimens

## Taxonomy

## Name, rank and serial number

## Biologists want to barcode half a million species in the next five years

THE tale of the unknown goby began in 1982 when Benjamin Victor, of the Ocean Science Foundation in Irvine, California, discovered an unusual fish in a reef in Panama. With only a single specimen he was hard pressed to prove it was a new species, so the fish remained, unnamed, on his desk for 25 years. Then, last year, he was sent an unusual fish larva. Using a new kind of DNA identification called barcoding he showed that it was a younger version of his mystery goby and that both specimens were, indeed, a new species.

DNA barcoding was invented by Paul Hebert of the University of Guelph, in Ontario, Canada, in 2003. His idea was to generate a unique identification tag for each species based on a short stretch of DNA. Separating species would then be a simple

which there are at least 3,500 species, many of them hard to tell apart.

So far Dr Linton's team has used the COI gene to distinguish 390 species of mosquito, of which 7% have turned out to be new species. *Anopheles oswaldoi*, for example, was known to be a carrier of malaria in northern, but not southern, Brazil. That was puzzling. DNA barcoding, however, has shown that *A. oswaldoi* is actually four species, of which only one carries malaria. That explains the geographical discrepancy and should also assist efforts to curb the disease in Brazil by allowing the real culprit to be studied in detail.

## Fly titles

The mosquito initiative has also had a piece of luck. Using some chemical wiz-

as medicines. In doing so, they have had to identify a new kind of barcode, as the COI gene is not found in plants.

Another group that could benefit from barcoding are customs officers, says Mark Blaxter, an evolutionary biologist at the University of Edinburgh. For those struggling to prevent the importation of pests or endangered wildlife, rapid and accurate identification tools are essential—particularly when perishable goods are being held up. America's Department of Agriculture is creating barcodes for the world's fruit flies. These are important agricultural pests and often arrive in the country as hard-to-identify larvae, or eggs, on fruit. Another group at the National Chung Hsing University in Taiwan (where hundreds of newly minted experts in the field have just met for the Second International Barcode of Life Conference) have created a prototype barcoding biochip. This is a collection of miniature DNA test sites on a sliver of glass that will rapidly discriminate between four species of fruit flies.

Barcoding's ease of use is also attracting interest from other government agencies. America's Federal Aviation Administration and its air force are working on bird

# July 2010 Technical Panel on Diagnostic Protocols Washington, DC

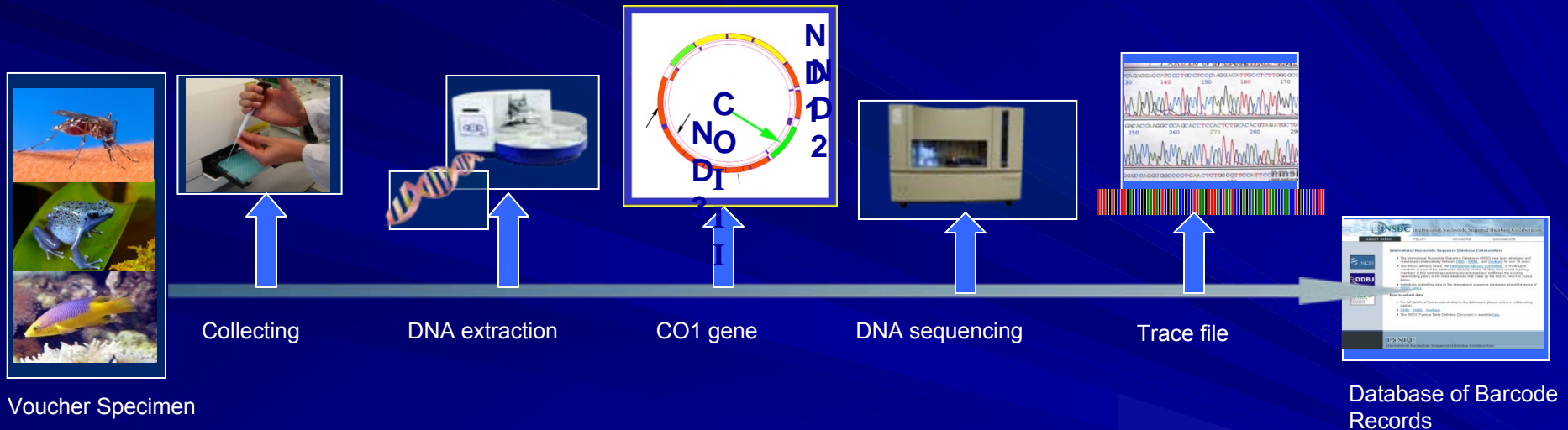


# How Barcoding Works

- First, build a barcode reference library:
  - Well-identified specimen
  - Tissue subsample
  - DNA extraction, PCR amplification
  - DNA sequencing
  - Data submission to GenBank
- Second, use it to identify unknowns:
  - Any unidentified juvenile, adult, fragment, product
  - Tissue sample, DNA, sequencing
  - Comparison with sequences in reference library

# The Barcoding Pipeline

## From specimen to sequence to species





# Current Norm: High throughput

## Large labs, hundreds of samples per day



ABI 3100 capillary  
automated sequencer

Large capacity PCR and  
sequencing reactions





- US\$100-165K purchase
- 150-500 samples per day
- 2-3 hours processing time
- US\$3-5 per sample

# Technology Development Partnership Goal

**The DNA  
Sequencing  
Lab of  
2013?**



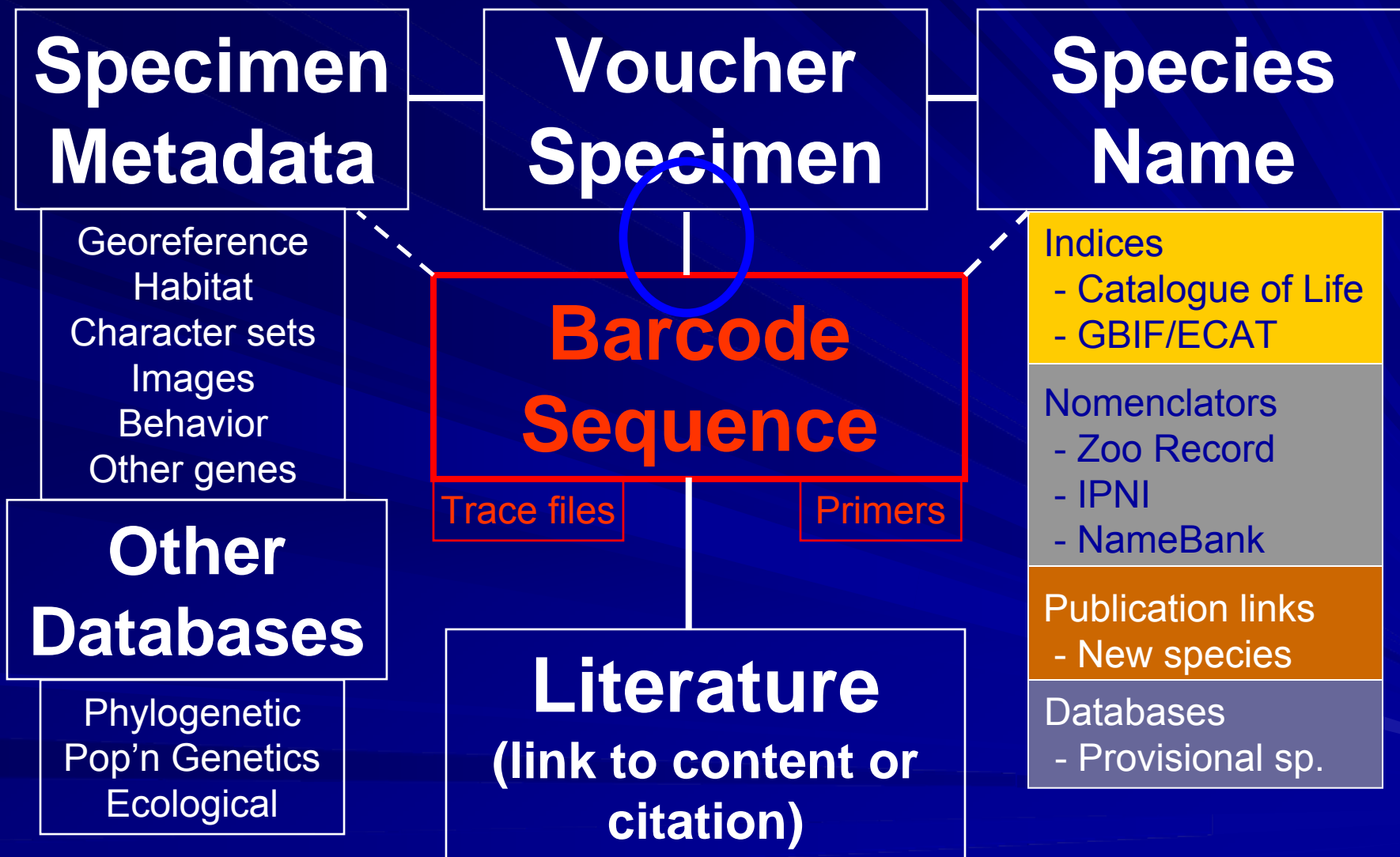
# Producing Barcode Data: 201?

Barcode data anywhere, instantly



- Data in seconds to minutes
- Pennies per sample
- Link to reference database
- A taxonomic GPS
- Usable by non-specialists

# BARCODE Records in INSDC



# 1 Million+ records, 100K+ species

**BOLD Systems - Specimen Record - Microsoft Internet Explorer provided by SINET**

http://www.boldsystems.org/connectivity/specimenlookup.php?processid=ABUAM086-07

**BOLDSYSTEMS** Management & Analysis

**UAM Marine Mammals [ABUAM]**

**Specimen Identifiers**

Sample ID :	UAM Mamm 86887	Museum ID :	86887
Field Num:	AF=50332	Collection Code :	Mamm
Deposited In :	University of Alaska Museum		
Publication :			
Donated By :			

**Specimen Details**

Voucher Type :	
Tissue Type :	
Extra Info :	
Sex :	

**Collection Data**

Collectors :  
Date Collected :  
Country :  
State/Province :  
Region/County :  
Sector :  
Exact Site :  
Latitude :  
Longitude :  
Coord. Source :  
Elevation/Depth :

**Photographs**

No images available

**Barcode Identifiers**

Barcode ID :	ABUAM086-07	Sample ID :	UAM Mamm 86887	Last Updated :	2007-04-12
Gene :	COX1	Translation Matrix :	Vertebrate Mitochondrial	GenBank Accession :	EU139289

**Sequencing Runs**

Run Date	Run Site	Direction	Trace File	PCR primers	Seq Pri
----------	----------	-----------	------------	-------------	---------

**Nucleotide Sequence**

Residues :	657	ACCCTATATTTACTATTTGGCCGCTGAGCGGGAATAGTAGGTACTGGTCTAAGCTTATTGATTGCTGCTGAATTAGGTCAACCTGGTACACTTATTGGAGACGAC
Comp. A :	177	CAGCTTTATAATGTTCTAGTAACAGCTCATGCCCTTCGTAATAATTTCTTTATAGTCATACCTATCATAATTGGAGGTTTGGAACTGATTAGTCCCTTAATA
Comp. G :	108	ATTGGAGCCCTGACATAGCATTCCCTCGTCTAAACAACATAAGCTTCTGACTACTTCCCCCTTCCCTCCTACTATTAATAGCGTCTTCGATAGTTGAAGCTGGC
Comp. C :	163	GCAGGTACAGGCTGAAGTGTATATCCCTCCTTAGCCGGAATCTAGCAGATCGAGGAGCCTCAGTAGACCTTACTATTTTCCCTACATTTAGCCGGCGTATCT
Comp. T :	209	TCAATCCTGGGGCTATTAAGTTCATTACAAGTATATTAATATAAAACCACCGCTATGACTCAATACCAACACCTCTCTTCGCTGATCAGTCCTTGTTACA
Ambiguous :	0	GCAACCTTACTTTTACTATCACTACCTGCTTAGCAGCCGGAATTAATACTATTGACTGATCGAAATCTAAACAACAACTTTTCGACCCGGCAGGAGGAGGG
		GATCCAATCTTATATCAACACTTATTC

**Amino Acid Sequence**

Residues :	219	TLYLLFGAWAGMVGTLGLSLIRAEALGQPGILIGDDQLYNLVIAHAFVMIFFMVMPIMIGGFNWLVPLMIGAPDMAFFPRLNMSFWLLPPSFLLLMASMVEAG
		AGTGWIVYPPLAGNLAHAGASVDLTIIFSLHLAGVSSILGAINFITTIINMKPPAMTQQYQPLFVWSVLVIATLLLLSLPVLAAAGITMLLIDRNLNTIFFDPAGGG
		DPILYQHLF

**Illustrative Barcode**

0 443  
444 656

# Sequence Webpages

**BOLDSYSTEM** | Management & Analysis
PRINT

Hesperiidae of the ACG 1 [CSCR]

### Barcode Identifiers

Barcode ID :	CSCR010-04	Sample ID :	
Gene :	COX1	GenBank Accession :	
Last Updated :		Translation Matrix :	

Model 377	13-TZBNA 238-03	Signal G:117 A:154 T:91 C:178
Version 3.3	BF1	DT377_BDv3_v2.mob
LR-377	TZBNA 238-03	214BDv3
Version 3.3.1b2	Lane 13	Points 1380 to 15200 Pk 1 Loc:

### Sequencing Runs

Run Date	Run Site	Direction	Trace File

### Nucleotide Sequence

Length :	617	NNAACTTTTATATTTTATTTTGGAAATTTGAGCAGGAATAGT
Comp. A :	203	TTAGGTAACCCAGGATCTTTAATTGGAGATGATCAAATTTA
Comp. G :	85	ATTTTTTTTATAGTAATACCAATTATAATTTGGAGGATTTGG
Comp. C :	92	GATATAGCATTTCCACGAATAAATAATATAAGATTTTGACT
Comp. T :	237	AGAAATGTAGAAAATGGAGCAGGAACAGGATGAACTGTTTA
Updated :	2005-09-09	TCCTCTGTAGACTTAGCTATTTTTTCATTACATTTAGCAGG
		ACAACAATTATTAATATACGAATTAGAAATTTATCATTGAC
		ACCGCACTTCTTTACTTTTATCTTTACCTGTTTTAGCTGG
		AATACATCATTCTTGGATCNNNNNNNNNNNNNNNNNNNNNN

### Amino Acid Sequence

Length :	220	XTLYFIFGIWAGMVGTSLSLLIRTELGNPGLIGDDQIYNT
		DMAFPMMNMSFWLLPPLMLLLISSIVENGAGTGWTVYVPLSANIAHQGSSVDLAIFSLHLAGISSILGAINFI
		TTIINMRISNLSFDQMPLFVWAVGITALLLLSLPVLGAIITMLLTDNRNLNTSFLDX-----

### Illustrative Barcode

# Specimen Webpages

**BOLDSYSTEMS** | Management & Analysis PRINT

*Hesperiidae of the ACG 1 [CSCR]*

**Specimen Identifiers** Edit Specimen

Sample ID :	02-SRNP-16276	Museum ID :	02-SRNP-16276
Isolate / Field Num:		Collection Code :	
Donated By :		Deposited In :	Smithsonian Institution

**Taxonomy**


Identifier :	18
phylum :	Arthropoda
class :	Insecta
order :	Lepidoptera
family :	Hesperiidae
subfamily :	Pyrginae
genus :	Anastrus
species :	Anastrus obscurus

**Specimen Details**

Voucher Type :	
Tissue Type :	
Extra Info :	Pyrginae
Sex :	m
Reproduction :	s
Life Stage :	



**Collection Data**

Collectors :	Roster Moraga
Date Collected :	12-Jul-2002
Country :	Costa Rica
State/Province:	Guanacaste
Region/County :	Area de Conservacion 276
Sector :	Del Oro
Exact Site :	Uncaria
Latitude :	11.0291
Longitude :	-85.4792
Coord. Source :	
Elevation/Depth :	300




**Photographs**

Dorsal View Ventral View



**BOLDSYSTEMS** | Management & Analysis PRINT

*Hesperiidae of the ACG 1 [CSCR]*



**BOLDSYSTEM** | Management & Analysis PRINT


*Hesperiidae of the ACG 1 [CSCR]*

**Specimen Identifiers**

Sample ID :	02-SRNP-16276	Catalog Number :	02-SRNP-16276
Isolate / Field Num:		Collection Code :	
Donated By :		Vouchered at :	2

**Photographs**

Dorsal View





# EOL Species Pages

HOME | PREFERENCES | LANGUAGE: EN | FEEDBACK | PRESS ROOM | USING THE SITE | ABOUT EOL

 [login](#) | [create an account](#)   SHOWING AUTHORITATIVE INFORMATION

Names  Tags  Full-text

---

## *Ostichthys kaianus* (Günther, 1880)

*Deepwater soldier*

Species recognized by [FishBase](#), R. Froese & D. Pauly (eds) in [Catalogue of Life](#)

IUCN RED LIST STATUS: **NOT EVALUATED**

CLASSIFICATION: [TEXT](#) | [GRAPHIC](#) | 

[Animalia](#) +  
[Chordata](#) +  
[Actinopterygii](#) +  
[Beryciformes](#) +  
[Holocentridae](#) +  
[Ostichthys](#) +  
[Ostichthys kaianus](#) (Günther, 1880)

[Archaea](#) +  
[Bacteria](#) +  
[Chromista](#) +  
[Fungi](#) +  
[Plantae](#) +  
[Protozoa](#) +  
[Viruses](#) +

**IMAGES**



COPYRIGHT: Some rights reserved  


SUPPLIER: [FishBase](#)   
SOURCE: [John E. Randall](#)

fish market; BPBM 10048 Locality: Nahs

# GenBank, EMBL, and DDBJ

## Global, Open Access to Barcode Data



ABOUT INSDC

POLICY

ADVISORS

DOCUMENTS



### International Nucleotide Sequence Database Collaboration

- The International Nucleotide Sequence Databases (INSD) have been developed and maintained collaboratively between [DDBJ](#) , [EMBL](#) , and [GenBank](#) for over 18 years.
- The INSDC advisory board, the [International Advisory Committee](#) , is made up of members of each of the databases' advisory bodies. At their most recent meeting, members of this committee unanimously endorsed and reaffirmed the existing data-sharing policy of the three databases that make up the INSDC, which is stated below.
- Individuals submitting data to the international sequence databases should be aware of [INSDC policy](#) .

### How to submit data

- For full details of how to submit data to the databases, please select a collaborating partner.
- [DDBJ](#) , [EMBL](#) , [GenBank](#)
- The INSDC Feature Table Definition Document is available [here](#) .

INSDC

International Nucleotide Sequence Database Collaboration

<http://www.insdc.org/>

# Link from GenBank to Museums

UAM Mamm 86887 - Microsoft Internet Explorer provided by SINET

http://arctos.database.museum/SpecimenDetail.cfm?GUID=UAM:Mamm:86887

Google

Go

Bookmarks

1 blocked

Check

AutoLink

AutoFill

Send to

Settings

lenovo

NCBI Sequence Viewer ...

UAM Mamm 86887

UAM Mamm 86887

Home

Preferences

Help

Site Map

Use Specimens

Collections

Log in

Some features of this site may not work in your browser. We recommend Firefox.



## Mammal Collection

University of Alaska Museum of the North

Specimen Search

Publication/Project Search

Advanced Features

UAM Mamm 86887

*Orcinus orca* Details

BerkeleyMapper

Oklee Spit, near Kayak Island

North America, United States, Alaska, Cordova Quad

28 Jul 2006

liver; heart; muscle; kidney

login or Create Account

Locality

North America, United States, Alaska, Cordova Quad

Oklee Spit, near Kayak Island

Lat/Long: 60° 3' 32" N 144° 10' 48" W ± 1 km Details

Collecting Date: 28 Jul 2006

Collectors

Tim Lebling

Preparators

Pam Tuomi

Used By: Canadian Barcode of Life Network

Parts:

liver; heart; muscle; kidney Details

Individual Attributes

Sex: unknown Details

Standard Measurements:

total length	tail length	hind foot	efn	weight
564 cm				


Remarks: Necropsy by Pam Tuomi ASKC.

Identifying Numbers

original identifier: 00-0602

AF: 50332

GenBank: EU139289



Mammal Collection

University of Alaska Museum at the University of Alaska Fairbanks, Fairbanks, AK 99775-6960.

System Administrator is Dusty McDonald.

Done

Internet

100%

# How Barcoding Works

- First, build a barcode reference library:
  - Well-identified specimen
  - Tissue subsample
  - DNA extraction, PCR amplification
  - DNA sequencing
  - Data submission to GenBank
- Second, use it to identify unknowns:
  - Any unidentified juvenile, adult, fragment, product
  - Tissue sample, DNA, sequencing
  - Comparison with sequences in reference library

# How Complete is the Barcode Library?

- More than 1 million records in BOLD
- More than 100,000 species represented
- Projects underway in all major groups
- Focus on groups with commercial and societal importance:
  - Agricultural pests
  - Disease vectors
  - Endangered species



# Barcode of Life Community



- Promote barcoding as a global standard
- Build participation
- Working Groups
- BARCODE standard
- International Conferences
- Increase production of public BARCODE records

## Networks, Projects, Organizations

international  
BARCODE  
OF LIFE

ECBOL

BOLDSYSTEMS

ABBI

FISH-BOL

BOLD

Canadian  
Barcode of Life  
Network

MBI  
Mosquito Barcoding Initiative

TB

CCDB

BIO

# Investments in Barcoding

- ~US \$5 million per year
  - Smithsonian Laboratories for Analytical Biology
  - Smithsonian barcoding projects
  - Sloan Foundation support for CBOL
  - Project support by USDA, EPA, FDA, FAA...
  - Barcoding in NSF-funded biodiversity grants

# Adoption by Regulators

- USDA, Belgian research projects on fruit flies
  - Plans for submission of Diagnostic Protocols
- Food and Drug Administration
  - Reference barcodes for commercial fish
- Environmental Protection Agency
  - \$250K pilot test, water quality bioassessment
- NOAA/NMFS
  - \$100K for Gulf of Maine pilot project
  - FISH-BOL workshop with agencies, Taipei, Sept 2007
- Federal Aviation Administration – \$500K for birds



# Investments in Barcoding

- ~US \$5 million per year
- CAN \$80 million over 2005-2015
- Commitments of ~CAN \$75 million from iBOL partners over 2010-2015
  - 5 million specimens
  - 500K species
  - 25 partner countries
  - Canada, US, EU, China are “central nodes”





# Consortium for the Barcode of Life (CBOL)

- Established May 2004 with Sloan Foundation grant
- Secretariat hosted by Smithsonian Institution
- Now in its fourth two-year funding period
- Workshops, Working Groups, networking, representation/marketing
- Now an international affiliation of 200+ members in 50+ countries:
  - Natural history museums, biodiversity organizations
  - Users: e.g., government agencies
  - Private sector biotech companies, database providers

# CBOL Member Organizations: 2010



- 200+ Member organizations, 50 countries
- 35+ Member organizations from 20+ developing countries

# Building the Community

- Internal communication through Community Network (<http://connect.barcodeoflife.net>)
- Outreach communication through
  - [www.barcodeoflife.org](http://www.barcodeoflife.org)
  - CBOL Webinars
- Coordination with other barcoding projects through CBOL's Implementation Board
- Steering Committee planning meetings
- Assistance in preparing and submitting proposals

# Connect.barcodeoflife.org

## connect.BarcodeofLife.net

international online community for dna barcoding professionals

[Home](#) [My Profile](#) [Members](#) [Groups](#) [Forum](#) [Blogs](#) [In the News](#) [FAQ](#) [Photos](#) [Videos](#)

This network allows DNA barcoding professionals in the field to discuss issues, share profiles, form special interest groups and more.

### Latest Activity



[Hosam Osama Elansary](#) is now a member of Connect.BarcodeofLife.net

[Welcome Them!](#)

on Monday



[Diego Pignataro](#) and [Ali Taheri](#) are now colleagues

on Sunday



[Marko Mutanen](#) is now a member of Connect.BarcodeofLife.net

[Welcome Them!](#)

on Friday



[Andrew Mitchell](#) and [richard stuart](#)

### Welcome to Connect.BarcodeOfLife.net

**New to our online community?** Here are few things you can do to get started:

- Complete your [profile](#)
- Add yourself to the [member map](#)
- Browse our [Forum](#) and ask a question or leave a comment
- Join a [Group](#) or start your own
- [Blog](#) about barcoding here or
- let us know if you'd like to share your own blog through our [rss pages](#)
- Have a question? Ask one of our [hosts](#) or Check out our [FAQ](#).

### Members



[View All](#)

Welcome to Connect.BarcodeofLife.net

[Sign Up](#)  
or [Sign In](#)

### About



[Matthew Fisher](#) created this Ning Network.

[Create a Ning Network! »](#)

### Translate

Select Language

Powered by [Google™ Translate](#)

### Poll

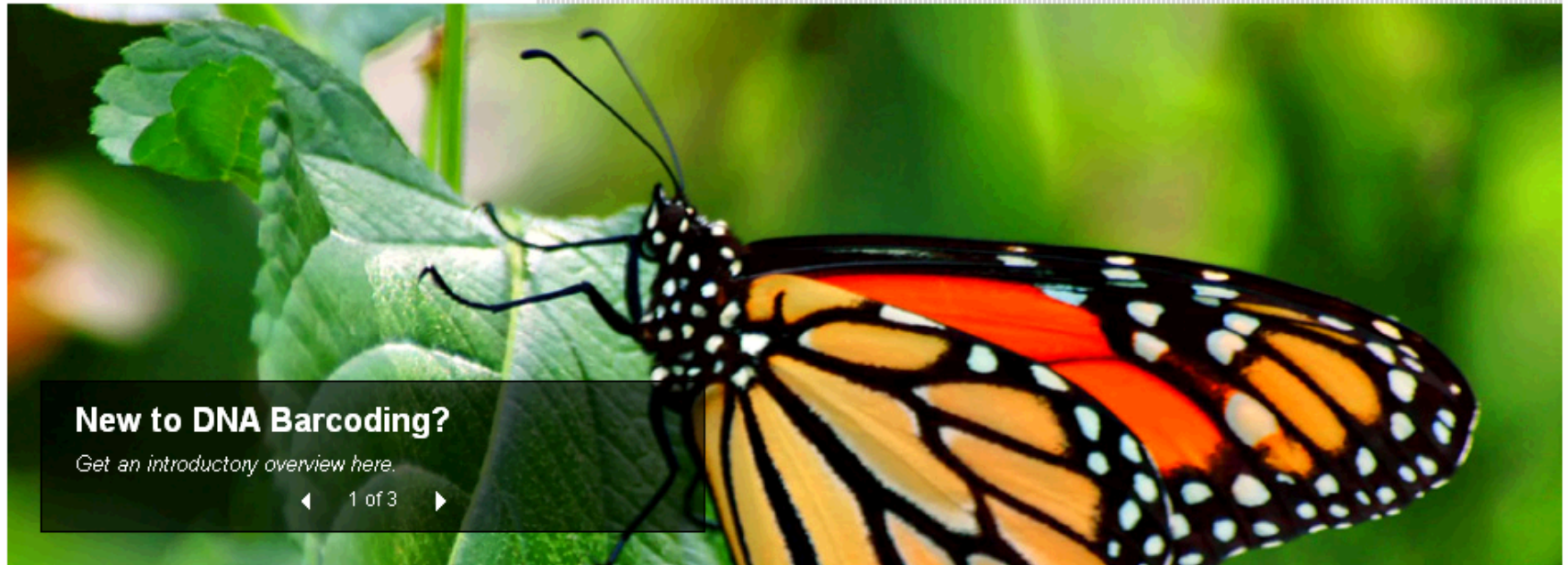
What would help you get the most out of Connect.BarcodeofLife.net?

- "How To" Tutorials to help you with the

## Barcode of Life

Identifying Species with DNA Barcoding

[About](#) [Community](#) [Resources](#) [Events](#) [Partners](#) [News](#)



### New to DNA Barcoding?

*Get an introductory overview here.*

◀ 1 of 3 ▶

#### PUBLICATIONS

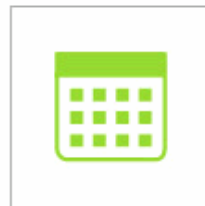


📄 **Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama**

Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O. and Bermingham, E., 2009. *The Proceedings of the National Academy of Sciences Online*. 106(44) 18621-6

📄 **Sex attractant, distribution and DNA barcodes for the Afrotropical leaf-mining moth *Phyllonorycter melanosparta* (Lepidoptera: Gracillariidae)**

#### EVENTS



2 JUNE 2010

### 2nd Conference of the European Consortium for the Barcode of Life (ECBOL2): 2010 International Year of Biodiversity

University of Minho, Braga, Portugal

[More Info](#)

[VIEW ALL](#)

#### COMMUNITY



[Hosam Osama Elansary](#) is now a member of [Connect.BarcodeofLife.net](#)  
[Welcome Them!](#)

2 days ago

[Diego Pignataro](#) and [Ali Taheri](#) are now colleagues

3 days ago

[Marko Mutanen](#) is now a member of

## Barcoding Projects

[Community](#)

[Barcoding Projects](#)

There are many international barcoding activities dedicated to the development of targeted public reference BARCODE sequence libraries.

### FEATURED PROJECTS



Sort By:



### All Birds Barcoding Initiative (ABBI)

ABBI, the All Birds Barcoding Initiative, is a campaign to collect DNA barcodes from 5 or more individuals of all of the approximately 10,000 bird species in the world. The ABBI DNA barcode library will help speed discovery of new species, open new avenues for scientific investigation, and provide a forensic tool for identifying specimens, including for example tissue fragments from bird-airplane collisions and avian blood samples from biting insects that harbor West Nile virus or other human disease agents.

[Project Site](#)



### All Fungi Barcoding

All Fungi Barcoding provides up-to-date information on fungal barcoding and facilitates communication and collaboration among researchers interested in fungi.

[Project Site](#)

# International Barcode Conferences

- Natural History Museum, London: 2005
- Academia Sinica, Taipei: 2007
- UNAM, Mexico City: 2009
- University of Adelaide, Australia: 2011
- All-Africa Conference: 2012
- 30-60 Travel Bursaries awarded for participants from developing countries



# Challenges

- Raising awareness about barcoding

# Outreach Activities

- Cape Town, South Africa, April 2006, SANBI
  - Scale insects in African agriculture
- Nairobi, Kenya, October 2006
  - Commercial fisheries in Rift Valley lakes
- Brazil, March 2007
  - Hardwood tree species
  - Endangered mammals, reptiles, amphibians
- Taiwan, September 2007
- Nigeria, October 2008
- Beijing, May 2009
- India, November 2010

# Challenges

- ✓ Raising awareness about barcoding
- Buy-in by national/international authorities
  - Access to study specimens for international research under the Convention on Biological Diversity



# DNA Barcoding:

A New Tool for  
Identifying Biological Specimens and  
Managing Species Diversity

# ABS Workshop, Museum Koenig

17-19 November 2008



# 51 Participants from 24 Countries

Sector		
Research	Agency	Other
29	10	12
56.9%	19.6%	23.5%

Geographic Representation				
OECD	Africa	Latin America	Asia	Pacific
28	8	4	9	2
54.9%	15.7%	7.8%	17.6%	3.9%

# COMMENT

**MID-TERMS** A two-year science to-do list for Obama p.781



**CLIMATE** Cost-benefit estimates of insurance are short on uncertainty p.784

**CLINICAL TRIALS** Inside the world of the professional human guinea pig p.788

**GENETICS** The inclusive father of gene-level selection remembered p.789

Nature magazine  
7 October 2010



A researcher prepares to analyse plant samples from the biodiversity-rich regions around Hanoi.

## Biology without borders

Fundamental research must not be hampered by an international agreement on sharing the benefits from national biodiversity, says David Schindel.

The supreme decision-making body of the United Nations Convention on Biological Diversity (CBD) meets in Nagoya, Japan, on 18–29 October 2010 for its tenth biennial conference. One of the most important items on the agenda is a new protocol which, if enacted, would specify how countries that are parties to the convention control access to their 'genetic resources' (including whole organisms, tissue samples and DNA extracts) and what benefits they can expect from sharing them. The negotiators' focus on genetic resources used to develop commercial products<sup>1</sup> has left non-commercial academic research in a perilous position<sup>2</sup>. One-size-fits-all legislation could have devastating effects on research conducted by foreign and local investigators, and even on the technological growth and economies of developing countries.

According to the CBD, countries can control access to their own species and set the terms for sharing any benefits resulting from their use by foreigners. Since 1993, only 15 of the 193 countries that have ratified the convention have passed legislation and created regulations to control access (another 58 have either legislation or regulations in place; see 'Where countries stand'). Most are hoping for a long-awaited international agreement to set global standards. The tenth Conference of the Parties (COP-10) could provide this.

Just last month, a CBD working group agreed on a new section to the draft protocol, proposed by the European Union and Japan. This directs CBD countries to encourage research that contributes to the conservation and sustainable use of biodiversity. More specifically, it directs them to create simplified access procedures for non-commercial research, with the understanding that mechanisms for handling unanticipated commercial applications may have to be developed. It is crucial that the parties to the convention approve the global access and sharing agreement only if this amendment is included.

### SOVEREIGN RIGHTS

The rich biological diversity of many developing countries has long attracted biologists interested in evolution and ecology, as well as researchers looking for compounds that could be developed into products such as drugs and cosmetics. Before the creation of the CBD, most government ministries didn't pay much attention to the collecting ▶

# CBD International Regime for Access and Benefit Sharing

In the development and implementation of their national legislation on access and benefit-sharing, [and on the basis of the sovereign right of Parties who regulate access to genetic resources and its derivatives,] Parties shall:

- (a) *Create conditions to **promote and encourage research** which contributes to the conservation and sustainable use of biological diversity, particularly in developing countries, including through **simplified measures on access for non-commercial research purposes**, taking into account the need to address a change of intent for such research*



# Challenges

- ✓ Raising awareness about barcoding
- Buy-in by national/international authorities
- Start-up funding
  - ✓ Mexican national barcoding network, equipment and project grants
  - ✓ Brazilian national funding program
  - ✓ India national initiative
  - ✓ South African national network

# Challenges

- √ Raising awareness about barcoding
- Buy-in by national/international authorities
- Start-up funding
- Training
  - √ CBOL training opportunities for researchers, students
  - √ Annual short courses: Buenos Aires, Johannesburg, Paris
    - Needs to be scaled up
    - Needs to be extended to regulatory officials, other users

# Challenges

- ✓ Raising awareness about barcoding
- Buy-in by national/international authorities
- Start-up funding
- Training
- Capacity building
  - Specimen repositories
  - Small labs for DNA extraction
  - National/Regional sequencing centers
  - Informatics capabilities



# Fourth International Barcode of Life Conference

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[ADELAIDE](#)

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Home



## Welcome

The [Consortium for the Barcode of Life](#) and the [University of Adelaide](#) invite you to join us in Adelaide, Australia from 28 November - 3 December 2011 for the Fourth International Barcode of Life Conference. Barcoding has seen extraordinary growth since the Mexico City Conference in November 2009 so join participants from around the world for the biggest barcoding event ever!

The organizers have developed this website to provide potential participants, co-sponsors, and other stakeholders with information about the conference. The conference organizers are also eager to have your feedback as we plan the conference so please share your ideas through [Connect, the DNA Barcoding network](#). You can do this by using the links found throughout this website.

## News Feed



Stay up to date by checking this webpage for new documents. You can also send us an [Expression of Interest](#) that will put you on an email distribution list, or sign up for an RSS feed by clicking on the icon above. Recently posted documents are:

### [Connect with the Fourth Conference: Blogs](#)

14/03/2011

In my last blog I introduced the Prepare for the Fourth Conference discussion page and encouraged users to ...

### [Connect with the Fourth Conference!](#)

04/03/2011

With key information on registration and abstract submission still in development and the conference more ...

## Important Dates

University of Adelaide  
South Australia  
28 November –  
3 December 2011



<http://www.dnabarcodes2011.org>