THIRD INTERNATIONAL

BARCODE OF LIFE

CONFERENCE

7 - 13 / NOV / 2009
MEXICO CITY

ABSTRACT
VOLUME

Welcome

The Consortium for the Barcode of Life (CBOL) and the Instituto de Biologia, Universidad Nacional Autonoma de Mexico (IB-UNAM) are proud to welcome you to Mexico City and the Third International Barcode of Life Conference. The Technical Program Committee and the Local Organizing Committee have prepared a week of plenary speakers, technical sessions, poster presentations, exhibits and social events and we hope you find it productive and stimulating. The Local Organizing Committee is especially eager to introduce you to the rapidly growing barcode community in Mexico and to help launch a vibrant set of barcoding projects in the regions.

DNA barcoding has come a long way since September 2007 when we met in Taipei for the Second International Barcode of Life Conference. CBOL has grown to have 200 Member Organizations from 50 countries, and the International Barcode of Life Project (iBOL) has attracted participants from around the world. Mexico has established MexBOL, a national barcoding network, and is positioned to be a regional node for iBOL. There are now more than 700,000 DNA barcode records representing more than 65,000 species in BOLD and data are accumulating at an accelerating pace.

This extraordinary progress means that the conference will be packed with information that people will want to exchange. You'll see that the presentations cover a great range of topics and taxonomic groups. Discussions will focus on projects that are far along, like FISH-BOL and TreeBOL, as well as new emerging projects that are barcoding endangered species, disease vectors, and protists. CBOL's Plant Working Group has made its long-awaited recommendation for the plant barcode region and the final decision will be announced at the conference.

The stage is set for an exciting, stimulating week in Mexico City. We hope you enjoy it!

| **David Schindel** | **Patricia Escalante** | **Scott E. Miller** |
| CBOL Executive Secretary | Conference Chairman | CBOL Chairman |

Table of Contents

Co-sponsors & Committees

Technical Program Committee

George Amato, American Museum of Natural History, USA
Ann Bucklin, University of Connecticut, USA
Robert de Salle, American Museum of Natural History, USA
Robert Hanner, BIO, University of Guelph, Canada
Peter Hollingsworth, Royal Botanic Garden Edinburgh, UK
Chris Meyer, Smithsonian Institution, USA
Sujeevan Ratnasingham, BIO, University of Guelph, Canada
David Schindel, Consortium for the Barcode of Life, USA
Kwang-Tsao Shao, Academia Sinica, Taiwan
Junko Shimura, Convention on Biological Diversity, Canada
Dirk Steinke, BIO, University of Guelph, Canada
Pablo Luis Tubaro, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Argentina
Cassio Van den Berg, Universidad Estadual De Feira De Santana, Brazil
Michelle Van der Bank, University of Johannesburg, South Africa

Local Organizing Committee

Instituto de Biología, UNAM, Mexico City	**Patricia Escalante Pliego, Chairperson**
	Gerardo A. Salazar Chávez
	Francisco Vergara Silva
	David Sebastian Gernandt
	Atilano Contreras Ramos
	Alejandro Zaldívar Riverón
	Roberto Garibay Origel
	Robert Bye Boettler
Instituto de Biología, UNAM, Colima	**Virginia Leon Règagnon**
	Ricardo Ayala Barajas
Instituto de Ciencias del Mary y Limnología (ICMyL), UNAM	**Adolfo Gracia Gasca**
Facultad de Ciencias, UNAM	**Joaquín Cifuentes Blanco**
El Colegio de la Frontera Sur (ECOSUR), Chetumal	**Manuel Elías Gutiérrez**
	Martha Valdéz Moreno
	Carmen Pozo de la Tijera
	Sergio Ticul Alvare
Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz	
Instituto de Ecología A. C	**Victoria Sosa Ortega**

Conference Program: Week-at-a-Glance

Pre-Conference Events

Saturday, 7-Nov	Sunday, 8-Nov	Monday, 9-Nov	
Workshop on Project Planning and Grant Proposal Writing **(Seminar Unit)**	Short Course on Barcoding Protocols **(Botanical Garden – Auditorium)**	**Morning**	Short course: data management in BOLD, GenBank, & BOLI Data Portal **(Seminar Unit)**
			Workshop on Barcoding Invasive Species **(Botanical Garden – Auditorium)**
Plant Working Group Meeting: **Morning** – GrassBoL **Afternoon** - TreeBoL **(Main Building – Video Conference Room)**	Plant Working Group Meeting **(Seminar Unit)**	**Afternoon**	IDRC Workshop on Barcoding in Developing Countries **(Botanical Garden Auditorium)**
			Data Analysis Working Group **(Main Building – Exam Room)**
			Advanced BOLD Workshop **(Seminar Unit)**
			Protist Barcoding Initiative **(Main Building – Video Conference Room)**
			Opening Reception, Ex Hacienda de Tlalpan

Conference Schedule

Tuesday, 10-Nov	Wednesday, 11-Nov	Thursday, 12-Nov
Session 1: Welcome and Introduction	**Session 3:** Case Studies: Impacts of barcode data in research areas beyond taxonomy	**Session 5:** Case Studies of Applications
Session 2: Planning Meso-American barcoding activities: lessons learned from 2004-2009	**Session 4:** Informatics and Data Analysis	**Session 6:** Barcoding and Next Generation Sequencing Technologies

Technical Sessions A and B	Technical Sessions C and D	Technical Sessions E and F
Plant Working Group _____ Marine Barcoding _____ Fish-BOL _____ Barcoding Pathogens, Disease Vectors & Parasites _____ All Birds Barcoding Initiatve & Vertebrates _____ Barcoding Species for Quarantine/Plant Protection	Plant Working Group/ Barcoding the Trees of Africa _____ Fish-BOL _____ Insects & Terrestrial Arthropods _____ Fungi/Algae/Protists/New Groups _____ Large-Scale Initiatives _____ Barcoding Databases, Protocols and Education _____ Data Analysis Working Group _____ BeeBOL Symposium	Meso-American Symposium _____ Canadian Network Business Meeting

Conference Program: Pre-Conference Workshops

Saturday, 7-November

Seminar Unit

Planning and Funding DNA Barcoding Projects

Organized by: The Global Taxonomy Initiative, Convention on Biological Diversity (GTI/CBD); The Consortium for the Barcode of Life (CBOL) & The International Barcode of Life Project (iBOL)

9:00am	Registration and refreshments
9:30am	Welcome; David Schindel, CBOL
	• Introduction of sponsors,
	• Preview of the Third International Barcode of Life Conference,
	• Goals of the workshop
9:45am	The Global Taxonomy Initiative of the CBD: Goals and Program of Work; Junko Shimura, GTI/CBD
10:15am	Overview: DNA barcoding, CBOL, Project Planning in Developing Countries; D. Schindel, CBOL
11:00am	Coffee break
11:30am	BioNET and CBOL Activities in East Africa; Beatrice Khayota, National Museums of Kenya
12:00	Results of Pre-workshop Questionnaire: Potential for barcoding projects in countries represented by workshop participants; D. Schindel
12:15pm	Plenary discussion: Plans and expectations for afternoon session
	• Types of projects and support from basic research to capacity-building
	• How to plan a barcoding project
	• How to write a grant proposal
12:30pm	Lunch in the UNAM Arboretum
2:00pm	Barcoding projects in the International Barcode of Life Project (iBOL); Robert Hanner, BIO, University of Guelph, Canada
2:45pm	What makes an effective barcoding grant proposal? Mock review of two proposals.
3:30pm	Coffee break
4:00pm	Funding from the Global Environmental Facility (GEF); B. Khayota, National Museums of Kenya; J. Shimura, GTI/CBD
4:30pm	Plenary discussion of potential projects identified by workshop participants
5:15pm	Summary of Workshop
6:00pm	Workshop adjourns, departure to hotels.

Homework for participants:

- Complete and return questionnaire
- Review background on DNA barcoding, guidelines for funding programs
- Read sample proposals and write 1-paragraph analysis and 1-paragraph recommendation on whether or not to fund the proposal
- Think about potential applications of DNA barcoding for local, national or regional priorities, and/or for academic research projects of interest
- Come prepared to focus on one potential barcoding project

Conference Program: Pre-Conference Workshops

Saturday, 7-Nov (9am- 12 Noon)
Video Conference Room

Plant Working Group Meeting: GRASS-BOL

Open meeting aimed at outlining priorities, identifying project participants and funding sources
for major project on grass barcoding.
[Organisers Hugh Cross (Hugh.Cross@sa.gov.au), Andy Lowe, Sean Graham].

9:00-9:20am	Introduction and overview of objectives and timelines of GrassBoL. [**Andy Lowe**]
9:20-9:40am	Overview of current taxonomic and phylogenetic status of grass family (Poaceae) and relatives (order Poales). [**Sean Graham, Hugh Cross**]
9:40-10:00am	Review of recent results for DNA barcoding of grasses. [**Hugh Cross**]
10:00-10:30am	Open discussion of alternative local barcodes and current grass genomic advances contributing to DNA barcoding of family. [Moderators: **Sean Graham, Hugh Cross**]
10:30-11:00am	Tea/Coffee. *Note: during the break, attendees who have interest in joining or contributing to project are encouraged to come forward and indicate their interest to meeting organizers. Mailing list will be compiled throughout the conference.*
11:00-12:00	Open discussion covering: organization of GrassBoL, addressing regional and taxonomic coverage of grass DNA barcoding; identifying both regional and international funding prospects. [Moderators: **Andy Lowe, Sean Graham**]

This is an open meeting and all interested participants are encouraged to come.

Saturday, 7-Nov (1:30 – 5.00 pm)
Video Conference Room

Plant Working Group Meeting: TREE-BOL

1:30-3:15pm	Open meeting with update presentations on TreeBoL progress.
3:45-5:00pm	Business meeting for regional chairs only[Organiser Damon Little: dlittle@nybg.org]

Sunday, 8-November
Botanical Garden Auditorium

Short Course on DNA Barcoding Protocols

9:00-9:15: Introduction to barcoding.   (Amy Driskell)
- What is the 'barcoding pipeline'?
- What data and object need to be tracked through the pipeline?
- What possible beginning and end points exist?

9:15-9:25: Barcode data standard   (David Schindel)

Part 1: Specimen Acquisition and Handling

9:25-9:30: Introduction to the issues   (Chris Meyer)
9:30-10:00: Museum Harvesting   (Rodolphe Rougerie & Alex Borisenko)
- Data quality: e.g. photographs, georeferences
- Destructive sampling
- Museum matters: vouchering, recataloging, archiving

10:00-10:30: Field Collection   (Sally Adamowicz and Chris Meyer)
- Legal issues: permitting, exporting
- Logistics
- Data quality and aquisition
- Changes to collection and preservation methods

10:30-11:00: Coffee break
11:00-11:10 Current Campaigns and Goals   (David Schindel)
11:10-12:30: Parallel groups for discussion and Q&A:
- Protists and fungi   (TBD)
- Plants   (Michelle Van Der Bank)
- Marine invertebrates   (Chris Meyer)
- Terrestrial invertebrates   (Rodolphe Rougerie)
- Vertebrates   (Alex Borisenko & Robert Hanner)

Part 2: Laboratory Methods and Data Management

2:00-2:45: DNA Extraction   (Dario Lijtmaer & Pablo Tubaro)
- Equipment
- Taxon- and target-specific methods
- Contamination
- Testing and quality control
- Storage and shipment of DNA extracts

2:45 -3:30: PCR amplification   (Oris Sanjur)
- Equipment, methods and reagents
- Taxon- and target-specific methods
- Contamination
- Testing and quality control

3:30 – 4:00: Coffee break
4:00 – 4:30: Information Management and data quality   (Amy Driskell)
- Tracking progress through laboratory pipeline
- Keeping all required products together
- Consistent data assessment
- Analysis-lab feed-back loop

Sunday, 8-November

Seminar Unit (9am–6pm)

General Plant Working Group Meeting

[Organiser **Pete Hollingsworth**; P.Hollingsworth@rbge.org.uk]

The aim of the meeting is to share information to develop and enhance the plant barcoding infrastructure. The emphasis of this meeting will be on open discussion/ information sharing, supported by very short presentations 5-10 mins. *If you have data which tackles any of the general issues on the agenda below and would like to discuss presenting the information at the workshop, please contact Pete Hollingsworth. Please note that full research talks should be presented at the main conference; presentations for the working group meeting will be aimed at facilitating discussion on development of plant barcoding infra-structure and protocols.*

A) PLANT BARCODING CURRENT STATUS

9:00- 10:00   Update and discussion on the CBOL recommend plant barcode [*this decision should be available from CBOL by the time of the conference*]

10:00-10:30   Inventory of ongoing plant barcoding projects, scale of activity/funding and contact details (a template will be prepared for participants to fill in before the meeting). Short presentations will be given on major barcoding campaigns that involve input from multiple laboratories (e.g. Tree-BoL, Grass-BoL; Mexican plants barcoding project)

10:30-11:00   Coffee break

B) DEVELOPING THE PLANT BARCODING INFRA-STRUCTURE

11:00-5:00   (lunch break from 12.30-1.30; coffee break from 3.30-4.00)

Improving the community resource for plant barcoding:

DNA Bank best practice (including discussion on storage and extraction protocols)

Core barcode, including discussions on:
- PCR and sequencing protocols for core-barcoding loci.
- Summary of amplification /sequencing successes/failures, target groups for further protocol development
- Development of mini-barcodes

Supplementary loci, including discussions on:
- Review of supplementary plastid barcodes
- Review of ITS for barcoding
- Development of guidelines for the use of supplementary loci
- Opportunities for accessing additional nuclear loci

Bioinformatics, including discussions on:
- Overview of available systems for plant barcoding
- Requirements of the plant barcode informatics work flow (quality checks, handling of data)
- Data analysis issues associated with use of two linked loci in DNA barcode
- Data analysis issues associated with the use of supplementary barcodes, including unlinked barcodes.

C) DELIVERING iBOL TARGETS

5:00-6:00   Discussion on iBoL priorities for plants.

Monday, 9-November
Seminar Unit

BOLD Short Course

Introductory session: This session is targeted as novice users but is a good refresher for infrequent users of BOLD. A maximum of 40 attendees will be accepted to allow for proper support during the hands-on training, It is highly recommended that this session be attended prior to the advanced course.

9:00am	Introduction and overview of general concepts BOLD, data structures and policies	**Sujeevan Ratnasingham**
9:30am	Data storage and management using the BOLD platform	**Megan Milton**
10:00am	Introduction to BOLD analysis tools.	**Sujeevan Ratnasingham**
10:20am	Project management and data sharing.	**Rodolpe Rougerie**
10:40am	Coffee break	
11:00am	Publishing data through BOLD and shared resources	**Taika von Kingslow**
11:20am	Hands-on training	
12:45pm	Lunch	

Advanced session: This session is targeted at users who have already have experience using BOLD to manage barcode data. It's highly recommended that individuals attending this session also attend the introductory session as a refresher.

BOLD Tools:

2:00pm	Welcome and introduction	**Sujeevan Ratnasingham**
2:20pm	Managing data quality in barcode workflows	**Evgeny Zakharov**
2:40pm	Data interpretation using BOLD analytics	**Sarah Adamowicz**
3:00pm	Managing multi-gene projects	**Alex Smith**
3:10pm	Integrating GIS tools	**Alex Smith**
3:30pm	Coffee break	
4:00pm	Hands-on training	

3rd Party Tools:

4:30pm	Overview of data exchange formats and web services for use with external tools	**Riadul Mannan**
4:40pm	Diagnostica: applications for barcode data	**Taika von Konigslow**
4:50pm	Visualizing amino acid variation in DNA barcodes	**Justin Schonfeld**
5:00pm	BOLI Data portal	**Neil Sarkar**
5:15pm	Closing remarks	**Sujeevan Ratnasingham**

Conference Program: Pre-Conference Workshops

Monday, 9-November
Botanical Garden – Auditorium

Uses of Barcoding and Genetic Markers for the Handling and Detection of Invasive Species

8:30 - 9:00	Registration
9:00 - 9:10	Welcome and introductions
9:10 – 9:30	Introduction
	Dr. Junko Shimura

1st session:

9:30am	Aquatic invasive species
	Roberto Mendoza, UANL, Mexico
9:50am	Identifying cryptic invasive ants in the Malagasy region
	Brian Fisher, CAS, USA
9:50am	*Arundo donax* and *Phragmites australis* in Mexico
	Erica Aguirre Planter, UNAM, Mexico
10:10am	DNA barcoding reveals cryptic invasive seaweeds in Canadian Coastal waters
	Gary Saunders, University of New Brunswick, Canada
10:30am	Barcoding migratory waterbirds as indicators of avian flu in Mexico
	Gary García, UNAM, México
10:50am	Coffee break

2nd session:

11:10am	DNA Barcoding of pest insects of importance to Australia
	Andrew Mitchell, Australia
11:30am	Detecting freshwater invasive leech species through the use of genetic bar codes
	Alejandro Oceguera-Figueroa & Mark E. Siddall, AMNH, USA
11:50am	Barcoding freshwater fish
	Martha Valdez, Ecosur, Mexico
12:10pm	"An example of barcode detection of an invasive: *Daphnia lumholtzi*, and the potential use of the barcodes in aquatic environments"
	Dr. Manuel Elías, ECOSUR, México
12:30- 1:00pm	Conclusions and end of meeting

Conference Program: Pre-Conference Workshops

Monday, 9-November
Botanical Garden –Auditorium

Barcoding of Life: Society and Technology Dynamics - Global and National Perspectives

Barcoding of Life: Society and Technology Dynamics - Global and National Perspectives
A symposium organized by the Canadian International Development Research Centre (IDRC)

2:00pm	Welcome, introduction of sponsor, and description of the goals of the workshop
2:15pm	CBD, the Global Taxonomy Initiative, and DNA barcoding in Developing Countries,
	Junko Shimura, CBD Secretariat, Montreal
2:30pm	"The Convention on Biological Diversity Access and Benefit Sharing Principles in the Context of Barcoded Genetic Information: The Case of iBOL",
	Manuel Ruiz Muller, Director of the Program of International Affairs and Biodiversity of the Peruvian Society for Environmental Law
3:00pm	"DNA Barcoding: Society and technology dynamics in the Indian context",
	Dr. Haribabu Ejnavarzala, Professor of Sociology, University of Hyderabad, India.
3:30pm	Coffee break
4:00pm	"iBOL as an Enabler of ABS and ABS as an Enabler of iBOL"
	Joseph Henry Vogel, University of Puerto Rico-Río Piedras
4:30pm	Consortium for the Barcode of Life (CBOL) activities in developing regions and with CBD
	David Schindel, Consortium for the Barcode of Life, Smithsonian Institution, USA
4:45pm	The International Barcode of Life Project (iBOL) and its relations to developing countries
	Paul Hebert, Biodiversity Institute of Ontario, University of Guelph, Canada
5:00pm	Questions and discussion by audience
5:45pm	Symposium ends
6:00pm	Conference registrants depart by bus for opening reception

Conference Program: Plenary Speakers & Technical Session Summaries

Tuesday, 10-November

Session 1: Welcome and Introduction

9:00-9:20	UNAM Official
	CONACYT official
9:20-9:25	**Ahmed Djoghlaf,** Convention on Biological Diversity
	Videotaped Welcome
9:25-9:45	KEYNOTE SPEAKER: **Antonio Lazcano** (UNAM)
	Natural history, microbes and sequences: back to the organism?
9:45-10:30	Panel discussion: Progress since Second International Conference
	Scott Miller, CBOL/Smithsonian (Moderator)
	Paul Hebert, iBOL
	Tila Maria Pérez Ortiz, MexBOL
	Pete Hollingsworth, Plant Barcoding
10:30-11:00	Coffee Break

Session 2: Planning Meso-American barcoding activities: Lessons learned from 2004-2009

11:00-12:00	Panel Discussion 2A: Strategies for Large Barcoding Initiatives
	Patricia Escalante, UNAM (Moderator)
	Sarah Adamowicz, BIO, University of Guelph
	Karen James, Natural History Museum London
	Mike Wilkinson, Aberystwyth University
12:00-12:45	Panel Discussion 2B: How has the Barcoding Paradigm improved Taxonomic Practices?
	Atilano Contreras, UNAM (Moderator)
	Andy Polaszek, Natural History Museum London
	Vazrick Nazari, BIO, University of Guelph
	Dan Janzen, University of Pennsylvania
12:45-2:00	Lunch

Parallel Technical Session A – 2:00-4:00

Plant Working Group	Auditorium A
Barcoding Pathogens, Vectors and Parasites	Auditorium B
Fish-BOL	Seminar Room A/B
Barcoding Species for Quarantine/Plant Protection	Seminar Room C
Marine Species	Seminar Room D
All Birds Barcoding Initiative and Vertebrates	Seminar Room E

Conference Program: Plenary Speakers & Technical Session Summaries

Parallel Technical Session B – 4:00-6:00

Plant Working Group	Auditorium A
Barcoding Pathogens, Vectors and Parasites	Auditorium B
Fish-BOL	Seminar Room A/B
Barcoding Species for Quarantine/Plant Protection	Seminar Room C
Marine Species	Seminar Room D
All Birds Barcoding Initiative and Vertebrates	Seminar Room E

Wednesday, 11-November

Session 3:   Case Studies:

Impact of barcode data in research areas beyond taxonomy

9:00-9:20	**Dario Lijtmaer,** MACN, Argentina
9:20-9:40	**C.J. Geraci,** Smithsonian
9:40-10:00	**Alex Smith,** BIO, University of Guelph
10:00-10:20	**Eske Willersley,** University of Copenhagen
10:20-10:40	Open discussion
10:40-11:10	Coffee Break

Session 4: Informatics and Data Analysis

11:10-11:30	**Neil Sarkar,** University of Vermont
11:30-11:50	**Sujeevan Ratnasingham,** BIO, University of Guelph
11:50-12:10	**Joaquín Giménez,** IBUNAM
12:10-12:30	**Kasper Munch,** Univ. California Berkeley
12:30-12:45	Open discussion
12:45-2:00	Lunch

Parallel Technical Session C – 2:00-4:00

Plant Working Group	Auditorium A
Data Analysis Working Group	Auditorium B
Fish-BOL	Seminar Room A
Large-Scale Initiatives	Seminar Room B
Fungi, Algae, Protists & New Groups	Seminar Room C
Insects/Terrestrial Arthropods	Seminar Room D
BeeBOL Symposium	Seminar Room E

Parallel Technical Session D – 4:00-6:00

Barcoding Databases, Protocols and Education	Auditorium A
Data Analysis Working Group	Auditorium B
Fish-BOL	Seminar Room A
Barcoding the Trees of Africa	Seminar Room B
Fungi, Algae, Protists & New Groups	Seminar Room C
Insects/Terrestrial Arthropods	Seminar Room D
BeeBOL Symposium	Seminar Room E

Conference Program: Plenary Speakers & Technical Session Summaries

Thursday, 12-November

Session 5: Case studies of Applications

9:00-9:20	**Phaedra Doukakis,** Stony Brook University
9:20-9:40	**George Amato,** American Museum of Natural History
9:40-10:00	**Daniel Masiga,** ICIPE, Nairobi
10:00-10:20	**Jeremey deWaard,** University of British Columbia
10:20-10:40	**Bernard Sweeney,** Stroud Water Center
10:40-11:10	Coffee Break

Session 6: Barcoding and Next Generation Sequencing Technologies

11:10-11:30	**Mehrdad Hajibabaei,** BIO, University of Guelph
11:30-11:50	**Tom Bruns,** Univ. California Berkeley
11:50-12:10	**Shadi Shokralla,** Biodiversity Institute of Ontario
12:10-12:30	**Michael Rhodes,** Applied Biosystems, California, USA
12:30-12:45	Open discussion

Parallel Technical Session E– 2:00-4:00

Meso-American Symposium	Auditorium A/B
Canadian Network Business Meeting	Seminar Room A/B

Parallel Technical Session F– 4:00-6:00

Meso-American Symposium	Auditorium A/B

Conference Program: Technical Sessions

Tuesday, 10-November

Session A

Plant Working Group (Chair: Sean Graham)	**Auditorium A**
2:00-2:15	**Aron Fazekas**, *University of Guelph* Patterns of plant species diversity below ground as revealed by DNA barcoding
2:15-2:30	**Andrew Lowe**, *University of Adelaide* Seeing the forest from the trees: Australian tree diversity
2:30-2:45	**Victoria Sosa**, *Instituto de Ecología, A.C.* An evaluation of multilocus dna barcodes in five mexican plant groups
2:45-3:00	**Harold Schneider**, *Natural History Museum, London* Utiltiy of plastid "barcodes" to identify plant species
3:00-3:15	**J.Y. Song**, *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China* Use of ITS2 region as universal barcode to identify medicinal plants and their adulterants
3:15-3:30	**Fernando Nicolalde-Morejón**, *Instituto de Ecología, A.C.* DNA barcoding in the Mexican cycads using CAOS
3:30-4:00	**Coffee Break**

Pathogens, Disease Vectors & Parasites (Chair: Virginia León Règagnon)	**Auditorium B**
2:00-2:15	**Scott Monks**, *U. Autónoma del Estado de Hidalgo* Potholes in the road to DNA barcodes for parasites
2:15-2:30	**Gabriela Parra-Olea**, *IBUNAM* Genetic studies of chytridiomycosis, an emerging infectious disease of amphibians
2:30-2:45	**Alejandro Oceguera-Figueroa**, *American Museum of Natural History* Use of DNA barcoding to detect invasive species and solve taxonomic problems within Hirudinea
2:45-3:00	**Mateus Pepinelli**, *Universidade de São Paulo* DNA barcoding highlights issues with morphology-based taxonomy of Neotropical black flies (Diptera: Simuliidae)
3:00-3:15	**Narendran Pradeep Kumar**, *Vector Control Research Centre Field Station (ICMR)* DNA barcoding of mosquitoes in India
3:15-3:30	**Yvonne-Marie Linton**, *Natural History Museum, London* Mosquito barcoding initiative: announcing the first data release paper
3:30-4:00	**Coffee Break**

Conference Program: Technical Sessions

Tuesday, 10-November

Session A, continued

FISH-BOL		Seminar Room A/B
2:00-2:15	**Richard Mayden**, *Saint Louis University*	
	DNA Barcoding and North American freshwater fishes	
2:15-2:30	**Luiz Henrique Pereira**, *Universida de Estadual Paulista (UNESP) – Botucatu, São Paulo, Brazil*	
	DNA barcode and the hidden diversity in the neotropical freshwater fishes	
2:30-2:45	**Erik García-Machado**, *Centro de Investigaciones Marinas, Universidad de la Habana*	
	DNA barcoding of cuban freshwater fishes: evidence for cryptic species and taxonomic conflicts	
2:45-3:00	**Daniel Carvalho**, *Universidade Federal de Minas Gerais*	
	DNA barcode of the fish species from the São Francisco river basin, Brazil	
3:00-3:15	**Claudio Oliveira**, *Universidade Estadual Paulista (UNESP) – Botucatu, São Paulo, Brazil*	
	Barcoding freshwater fishes from upper Parana basin	
3:15-3:30	**Jefferson Henriques**, *Universidade Estadual Paulista - UNESP*	
	Barcoding freshwater fishes from ribeira de Iguape basin	
3:30-4:00	**Coffee Break**	

Barcoding Species for Quarantine/Plant Protection (Chair: Isabel González)	Seminar Room C
2:00-2:15	**Introduction**
2:15-2:30	**Andrew Mitchell,** *NSW Department of Industry and Improvement*
	Mind the gap: Barcodes and diagnostic standards
2:30-2:45	**Laura Boykin**, *Bio-Protection Research Centre*
	Realizing the scope for barcodes in quarantine
2:45-3:00	**Wen Chen**, *Carleton University*
	DNA barcodes for the profiling of microbiota in environmental samples
3:00-3:15	**Adeniyi Akanni Jayeola**, *University of Ibadan*
	Selecting priority trees for DNA barcoding in Africa
3:15-3:30	**Rebecca Nakacwa**, *National Agricultural Research Laboratories*
	Soil nematode diversity and community composition as a basis for biosafety assessment of transgenic
3:30-4:00	Coffee Break

Marine Barcoding	Seminar Room D
2:00-2:15	**Ann Bucklin,** *University of Connecticut*
	DNA barcoding of marine zooplankton: current status and applications for ecosystem monitoring
2:15-2:30	**Leo Blanco-Bercial**, *University of Connecticut*
	Global phylogeographies of the planktonic copepod Clausocalanus based on DNA barcodes
2:30-2:45	**Adriana Radulovici**, *University of Quebec at Rimouski*
	Marine Crustaceans identified by DNA barcodes
2:45-3:00	**Paola Batta Lona**, *University of Connecticut*
	Applications of DNA barcodes for studies of Antarctic krill: SS-PCR and mitochondrial SNPs
3:00-3:15	**Laetitia Plaisance**, *Smithsonian Institution National Museum of Natural History*
	Standardized sampling and DNA Barcoding for Assessing Coral Reef Biodiversity
3:30-4:00	Coffee Break

Tuesday, 10-November

Session A, continued

All Bird Barcoding Initiative & Vertebrates	Seminar Room E
2:00-2:15	**Mariana Lyra,** *Universidad Estadual de Campinas*
	Barcoding anurans from Brazilian Atlantic forest
2:15-2:30	**Jing Che**, *State Key Laboratory of Molecular Evolution and Genome Diversity*
	Barcoding amagid lizards in southern China and Vietnam
2:30-2:45	**Kevin Kerr**, *BIO, University of Guelph*
	DNA barcode resolution in eastern Palearctic birds
2:45-3:00	**Pilar Benites**, *Museo Argentino de Ciencias Naturales*
	Neotropical birds barcoding: a joint initiative
3:00-3:15	**Leonardo Campagna**, *Museo Argentino de Ciencias Naturales*
	COI flags a recen radiation in passerine birds
3:15-3:30	**Patricia Escalante**, *Instituto de Biologia Universidad Nacional Autonoma de Mexico*
	Highlights of the Birds of Mexico project
3:30-4:00	Coffee Break

Conference Program: Technical Sessions

Tuesday, 10-November

Session B

Plant Working Group (Chair: Gerardo Salazar) Auditorium A	
4:00-4:15	**Mike Wilkinson**, *Aberystwyth University* Compiling and exploiting a national barcode for Wales
4:15-4:30	**Wendy Clement**, *Yale University* Barcoding the woody angiosperm clade Viburnum
4:30-4:45	**Sribash Roy**, *National Botanical Research Institute, Lucknow, Uttar Pradesh, India* Plant dna barcoding and concept of a universal locus may not work in complex group: a case study with *Berberis*
4:45-5:00	**David Gernandt**, *Instituto de Biología, Universidad Nacional Autónoma de México, México DF, México* Genealogical nonmonophyly in *Pinus* and its relevance for DNA barcoding
5:00-5:15	**Diana Percy**, *University of British Columbia* Plant barcoding in taxonomically complex groups: grasses and willows
5:15-5:30	**Hugh Cross**, *State Herbarium of South Australia* Leaves of grass: barcoding an iconic and enigmatic plant family

Pathogens, Disease Vectors & Parasites (Chair: Yvonne-Marie Linton) Auditorium B	
4:00-4:15	**Gerardo Pérez Ponce de León**, *IBUNAM* Molecular prospecting for cryptic species of parasites: are DNA barcodes useful?
4:15-4:30	**Sean Locke**, *Concordia University* Barcoding advances freshwater fish parasitology
4:30-4:45	**David Marcogliese**, *Environment Canada* Do we need barcodes for the parasitic helminths?
4:45-5:00	**Hugo Mejía Madrid**, *CINVESTAV- Mérida* Nematode diseases: the plurality of DNA barcoding
5:00-5:15	**Virginia León Règagnon**, *IBUNAM* Barcodes of helminths of wild vertebrates in Mexico
5:15-5:30	**Daniel Brooks**, *University of Toronto* Genetic Barcoding and the evolutionary ecology of emerging diseases

FISH-BOL Seminar Room A/B	
4:00-4:15	**G.D. Khedkar**, *Dr. Babasaheb Ambedkar Marathwada University* DNA barcoding reveals a discontinuous genetic diversity pattern of fish in the Godavari river, India
4:15-4:30	**Rosalee Rasmussen**, *Oregon State University* DNA barcoding of commercially important salmon and trout species in North America
4:30-4:45	**Dickens Odeny**, *National Museums of Kenya* DNA barcoding: refining parataxonomy for fishery surveys
4:45-5:00	**Nina Bogutskaya**, *Zoological Institute, Russian Academy of Sceinces* Barcoding of the freshwater fish fauna of Russia: a pilot project
5:00-5:15	**Julien April**, *Université Laval* Barcoding freshwater fishes: extensive coverage and sub-specific identification
5:15-5:30	**Jonathan Banks,** *Department of Biological Sciences, University of Waikato* Biosurveillance of fish and zooplankton in New Zealand lakes and reservoirs using DNA barcoding

Tuesday, 10-November

Session B, continued

Barcoding Species for Quarantine/Plant Protection (Chair: Marc de Meyer)	**Seminar Room C**
4:00-4:15	**Vazrick Nazari**, *BIO, University of Guelph* Coleophora (Lepidoptera, Coloephoridae): enhanced species discovery and taxonomy through DNA barcoding
4:15-4:30	**Antonio Hernández López**, *Institut National de la recherché Agronomique (INRA)* Host tracking, cryptic adaptation? A barcode study of the parasitoid of the horse chestnut leafminer
4:30-5:00 (Posters)	**Badrul Bhuiya**, *University of Chittagong* DNA barcoding of Agromyzid leaf miners and their parasitoids in Bangladesh
	Nelson Ntonifor, *University of Buea* Arboreal ant species as bio-control agents of pests
	Andrew Mitchell, *NSW Department of Industry and Investment* Comprehensive barcoding of Australian Heliothine moths

Marine Barcoding	**Seminar Room D**
4:00-4:15	**Francis Xavier Kidangan**, *National Institute of Oceanography* Molecular systematic of prawns under the family *Penaeidae* of Indian coast
4:15-4:30	**Tyler Zemlak**, *Dalhousie University* The intelligent observer: using the Barcode of Life Database to investigate the role of the OXPHOS system
4:30-4:45	**Julien Lorion**, *Museum National d/Histoire Naturelle* Marine invertebrates and the "barcode factory" at the Museum National d/Histoire Naturelle
4:45-5:00	**A. Biju Kumar,** *University of Kerala* Molecular taxonomy of putrefied Cetaceans- A case study

All Bird Barcoding Initiative & Vertebrates	**Seminar Room E**
4:00-4:15	**Brendan Reid**, *American Museum of Natural History* Distance-based and character-based approaches to barcoding turtles
4:15-4:30	**Elizabeth Clare**, *BIO, University of Guelph* Unraveling the food web of an insectivorous bat community
4:30-4:45	**Cristina Miyaki**, *Universidad de Sao Paulo* DNA barcodes against the illegal parrot trade
4:45-5:00	**Matthew Miller,** *Smithsonian Tropical Research Institute* New tools to study speciation and community ecology: examples from Panama's bird barcodes
5:00-5:15	**Mark Stoeckle**, *Rockefeller University* Digital indicator vectors map genetic diversity in South and North American birds

Wednesday, 11-November

Session C

Plant Working Group (Chair: David Gernandt)	**Auditorium A**
2:00-2:15	**Santiago Madriñán,** *Universidad de los Andes* ArBOL: A DNA barcoding initiative for neotropical plants
2:15-2:30	**Rolando B**árcenas, *Universidad Autonoma de Queretaro* DNA barcodes could help to identify and conserve Mexican Cactaceae
2:30-2:45	**G. Salazar**, *Instituto de Biología, UNAM, D. F., Mexico* DNA barcodes of Mexican oaks
2:45-3:00	**Ramalingham Sathishkumar**, *Bharathiar University* Conventional and novel DNA barcodes for *Apocyanaceae*
3:00-3:15	**Chang Liu**, *University of Hong Kong* The psbA-trnH intergenic spacer region database-a web service for dna barcoding
3:15-3:30	**Karen James**, *Natural History Museum, London* Project BarkCode: engaging schools in Tree-BOL
3:30-4:00	Coffee Break

Insects/Terrestrial Arthropods: Utility & Alternative Approaches	**Auditorium B**
(Chair: Atilano Contreras-Ramos)	
2:00-2:15	**Rodolphe Rougerie**, *BIO, University of Guelph* DNA barcoding Lepidoptera: what beyond taxonomy?
2:15-2:30	**Massimiliano Virgilio**, *Royal Museum for Central Africa* Performance of DNA barcoding for insect identification
2:30-2:45	**Julio Rivera**, *Universidad Nacional Agraria* DNA barcoding and the phylogeography of the blackfly *Prosimulium travisi* (Diptera: Simuliidae)
2:45-3:00	**Johannes Bergsten,** *Swedish Museum of Natural History, Stockholm,* Geographical scale of sampling and dna barcoding
3:00-3:15	**P. Pedro**, *BIO, University of Guelph* Degree of 28s rDNA divergence in Costa Rican barcoded Lepidoptera
3:15-3:30	**Michael Raupach**, *Zoologisches Forschungsmuseum Alexander Koenig* Molecular taxonomy of ground beeltes (Insecta: Carabidae) in Central Europe: a multi-marker approach
3:30-4:00	Coffee Break

Conference Program: Technical Sessions

Wednesday, 11-November

Session C, continued

FISH-BOL		Seminar Room A
2:00-2:15	**Martha Valdez-Moreno,** *El Colegio de la Frontera Sur* Advance with fish barcodes in Mexico	
2:15-2:30	**Juan Díaz de Astarloa**, *Departamento de Ciencias Marinas, Universidad Nacional de Mar del Plata* Barcoding Argentine marine fishes	
2:30-2:45	**Wazir Lakra**, *National Bureau of Fish Genetic Resources* DNA Barcoding The Indian Fishes	
2:45-3:00	**Agnes Dettai**, *Museum National D'histoire Naturelle* The CEAMARC survey: Barcodes as a multi level t	
3:00-3:15	**Yuri Kartavtsev**, *A.V. Zhirmunsky Institute of Marine Biology of the FEB RAS* Sequence divergence at CO-1 and Cyt-B mtDNA on different taxonomic levels and genetics of speciation and phylogenetics	
3:15-3:30	**Kwang-Tsao Shao**, *Biodiversity Research Center, Academia Sinica* Accuracy of morphological identification of larval fishes	
3:30-4:00	Coffee Break	

Large-Scale Initiatives		Seminar Room B
2:00-2:15	**Cara Gibson**, *The National Ecological Observatory Network (NEON)* The NEON Fundamental Sentinel Unit: ORganismal measurements and DNA barcoding in a national network	
2:15-2:30	**Jesus Mavarez**, *Centro de Ecología, Instituto Venezolano de Investigaciones Científicas* GenoMaps: DNA Barcoding applied to a large-scale biodiversity monitoring initiative in South America	
2:30-2:45	**Laura Epp**, *National Center for Biosystematics (NCB), Natural History Museum, University of Oslo* BARFROST- A new project for reconstructing past ecosystems by barcoding DNA from permafrost	
2:45-3:00	**Vincent Opyene**, *Uganda Wildlife Authority* Application of DNA in bushmeat barcoding in prosecution of wildlife crimes in East Africa	
3:00-3:15	**Neil Davies**, *University of California, Berkeley* International Ecostations Journal: BioIP Management Solution	
3:15-3:30	Discussion	
3:30-4:00	Coffee Break	

Conference Program: Technical Sessions

Wednesday, 11-November

Session C, continued

Fungi, Algae, Protists & New Groups (Chair: Pedro Crous)	Seminar Room C
2:00-2:15	**Wieland Meyer**, *University of Sydney* Barcoding of medical fungi- its region and its limitations
2:15-2:30	**Roberto Garibay-Orijel**, *Universidad Nacional Autonoma de Mexico (UNAM)* The framework to barcode neotropical ectomycorrhizal fungi
2:30-2:45	**Matteo Garbelotto**, *University of California, Berkeley* Biocoding the fungi of Moorea
2:45-3:00	**Agathe Vialle**, *Centre d'Etude de la Forêt* DNA Barcoding of fungal species from leaf of poplars
3:00-4:00	Coffee Break

Data Analysis Working Group (DAWG) (Chair: Neil Sarkar)	Seminar Room D
2:00-2:18	**Melanie Lou,** *McMaster University* Assigning sequences to species in the absence of a barcoding gap
2:18-2:36	**John Wilson**, *BIO, University of Guelph* Assigning unknowns to higher taxa using DNA barcodes
2:36-2:54	**Catherine Laredo**, *INRA, Mathématiques et informatique appliquées* Error rates of phylogenetic and supervised classification algorithms in DNA Barcoding
2:54-3:12	**Giovanni Felici**, *Istituto di Analisi dei Sistemi ed Informatica Consiglio Nazionale delle Ricerche* The BLOG system: Logic data mining for compact explanatory species classification
3:12-3:30	**Pavel Kuksa**, *Rutgers University* Efficient alignment-free barcode analytics
3:30-4:00	Coffee Break

BeeBol Symposium	Seminar Room E
2:00-2:05	**Ricardo Ayala**, *IBUNAM* Introduction
2:05-2:20	**Laurence Packer**, *York University* The campaign to barcode the bees of the world: progress, problems, prognosis
2:20-2:35	**Jason Gibbs**, *York University* DNA barcoding a nightmare taxon: a case study from bees
2:35-2:50	**Nelly Ndungu**, *International Centre for Insect Physiology and Ecology* Morphometrics and dna barcoding of stingless bees (apidae: meliponinae) in three selected forests in Kenya
2:50-3:05	**Ricardo Ayala**, *IBUNAM* The native bees of México and the DNA Barcode of Life project
3:05-3:20	**Seán Brady**, *National Museum of Natural History* Applying DNA barcoding and morphology toward improving the taxonomy of the cleptoparasitic bee genus *Nomada*
3:20-3:35	**Sheila Dumesh**, *York University* Barcoding bees with emphasis on Canadian Megachile: implications, resolutions and new associations
3:35-4:00	Coffee Break

Conference Program: Technical Sessions

Wednesday, 11-November

Session D

Barcoding the Trees of Africa (Chair: Helida Oyieke)	Auditorium A
4:00-4:15	**Olivier Maurin**, *University of Johannesburg* Explaining tree and shrub regional diversity patterns in the Kruger National Park (South Africa)
4:15-4:30	**Michelle van der Bank**, *University of Johannesburg* Strengthening Africa's capacity in DNA technologies for biodiversity research and sustainable use
4:30-4:45	**Yalemtseha Mekonnen Tadesse**, *Addis Ababa University* Strengthening TreeBOL Africa initiative
4:45-5:00	**Paul T. Lyam**, *National Center for Genetic Resources and Biotechnology (Nacgrab)* Barcoding threatened plant species of West Africa– Nigeria as a case study
5:00-5:15	**O.T. Ogundipe**, *University of Lagos* Tree BOL Lagos: barcoding of tres in Lagos

Insects/Terrestrial Arthropods: Biodiversity Studies (Chair: Alejandro Zaldívar-Riverón)	Auditorium B
4:00-4:15	**Rodolphe Rougerie**, *BIO, University of Guelph* DNA barcoding of archival Lepidoptera specimens
4:15-4:30	**Xin Zhou**, *BIO, University of Guelph* Trichoptera Barcode of Life: probing caddisfly diversity with DNA barcodes
4:30-4:45	**Birthe Thormann**, *Zoologisches Forschungsmuseum Alexander Koenig* Testing a short nuclear barcode for inferring staphylinid beetle diversity in an African rainforest
4:45-5:00	**Brian Fisher**, *California Academy of Sciences* Identifying cryptic invasives in the Malagasy region
5:00-5:15	**Facundo Labarque**, *Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"* Inferring biodiversity patterns and life-history traits in ray spiders (Araneae, Theridiosomatidae)
5:15-5:30	**Jonathan Clark**, *Weber State University* DNA barcoding of shore flies from Great Salt Lake

FISH-BOL	Seminar Room A
4:00-4:15	**Nicolas M Hubert**, *Ephe-Université de Perpignan* Identifying early stages of coral reel fishes through dna barcoding: a test case with the families Acanthuridae and Holocentridae
4:15-4:30	**Jonathan Deeds**, *US Food and Drug Administration Center for Food Safety and Applied Nutrition* US FDA Validation of DNA Barcoding to Promote Seafood Safety and Combat Economic Adulteration
4:30-4:45	**Dirk Steinke ,** *BIO, University of Guelph* Barcodes and mitochondrial protein evolution in fishes
4:45-5:30	Open Discussion

Conference Program: Technical Sessions

Wednesday, 11-November

Session D, continued

Barcoding Databases, Protocols and Education	Seminar Room B
4:00-4:15	**Robert Hanner**, *BIO, University of Guelph* Bio-pedagogy and Barcoding: The Canadian National Market Survey
4:15-4:30	**Evgeny Zakharov**, *BIO, University of Guelph* Quality assurance and crowd control in a high-throughput DNA barcoding facility
4:30-4:45	**Alex Borisenko**, *BIO, University of Guelph* A template for field for field data collection to aid
4:45-5:00	**Juncai Ma**, *Institute of Microbiology, Chinese Academy of Sciences* The Information System of DNA Barcode of Life in China
5:00-5:15	**Natalia Ivanova**, *BIO, University of Guelph* Protocols for dry DNA storage and shipment at room temperature
5:15-5:30	**Thomas Knebelsberger**, *Zoologische Staatssammlung München* Beyond barcoding – Secure DNA storage

Fungi, Algae, Protists & New Groups (Chair: Line Le Gall)	Seminar Room C
4:00-4:15	**Gary E. Saunders**, *University of New Brunswick* Molecules versus morphologies – a contemporary floristic survey of Canadian seaweeds
4:15-4:30	**David Porco**, *BIO, University of Guelph* Barcoding invasives: a new tool for invasion monitoring in soil
4:30-4:45	**Rina Ramírez**, *Museum of Natural History, San Marcos University* Barcoding orthalicid land snails from Peru

Data Analysis Working Group (DAWG) (Chair: Neil Sarkar)	Seminar Room D
4:00-4:18	**Mark Stoeckle**, *Rockefeller University* Digital indicator vectors reveal discontinuous genetic structure of biodiversity
4:18-4:36	**Taika von Königslöw**, *BIO, University of Guelph* A tool for identifying diagnostic DNA characters
4:36-4:54	**Raúl Jiménez-Rosenberg**, *CONABIO* Linking DNA barcodes and biodiversity information: the biodiversity information system of Mexico
4:54-5:12	**Mihai Albu,** *Concordia University* The DNA Barcode Linker
5:2-5:30	**Justin Schonfeld**, *BIO, University of Guelph* Visualizing amino acid variation in the COI barcode region

Wednesday, 11-November

Session D, continued

BeeBOL Symposium	Seminar Room E
4:00-4:15	**Robert Paxton**, *Queen's University Belfast* Barcoding reveals cryptic bumble bee species diversity
4:15-4:30	**Carmen Lucia Yurrita**, *Universidad de San Carlos de Guatemala* The bee fauna of Guatemala and the importance of the participation in the barcode of life
4:30-4:45	**Nicholai de Silva**, *York University* Estimating diversity: DNA barcoding and morphospecies
4:45-5:00	**Harrison Kibogo**, *International Centre of Insect Physiology and Ecology* Molecular characterization of Honeybees (*Apis mellifera*) races from Kenya using barcoding markers
5:00-5:15	**Jose Javier Quezada-Euan**, *UNAM* Pollinators at risk: identifying cryptic species in native bees from mexico (Hymenoptera:Meliponini)
5:15-5:30	**Miriam Richards**, *Brock University* Niche partitioning based on nesting biology in twig-nesting carpenter bees revealed by congruent variation in behavior, morphology, and DNA barcodes.
5:30-6:00	Open Discussion

Conference Program: Technical Sessions

Thursday, 12-November

Session E

Meso-American Symposium Auditorium A	
2:00-2:15	**Manuel Elías-Gutiérrez**, *ECOSUR Chetumal* MEXBOL, the Mexican commitment to DNA barcodes
2:15-2:30	**Patricia Escalante**, *IBUNAM* Development of the academic node of MexBOL at the institute of biology of UNAM
2:30-2:45	**Robert Bye**, *IBUNAM Botanical Garden* Complimentarily and challenges of dna bar codes for taxanomic determination of useful plants
2:45-3:00	**Virginia León Règagnon**, *IBUNAM Chamela* All Taxa Barcoding Initiative in Chamela-Cuixmala, México
3:00-3:15	**Martha Valdéz-Moreno**, *ECOSUR Chetumal* FISHBOL strategies in Meso America
3:15-3:30	**Adolfo Gracia**, *Brock University* Crustacean decapods of the Gulf of Mexico, superfamily Penaeoidea
3:30-4:00	**Coffee Break**

Session F

Meso-American Symposium	
4:00-4:15	**Joaquín Cifuentes**, *Facultad de Ciencias UNAM* Complementing different macrofungi campaigns: taxonomic and regional inventory plus functional group biodiversity studies
4:15-4:30	**Andrew Polaszek**, *Natural History Museum, London* Barcoding megadiverse *Encarsia* parasitoids in Mexico
4:30-4:45	**Patricia Landaverde**, *Universidad de San Carlos de Guatemala* Biodiversity of Guatemala and the importance of participating in the barcode of life
4:45-5:00	**Lucia Páiz-Medina**, *Universidad Centroamericana* DNA barcode of Midas Cichlidae species complex inhabiting lakes and lagoons of Nicaragua
5:00-5:15	**Daniel Janzen**, *University of Pennsylvania* Barcoding a very complex tropical trophic food web

Plenary Session Speakers & Abstracts

Session 1: Welcome and Introduction

KEYNOTE SPEAKER: Antonio Lazcano

Antonio Lazcano, a professor at the Universidad Nacional Autónoma de México (UNAM) in Mexico City, has studied the origin and early evolution of life for over 30 years. He was trained both as an undergraduate and graduate student at the UNAM, where he rapidly focused on the study of prebiotic evolution and the emergence of life. An academic deeply committed to public education, he has also devoted considerable efforts to scientific journalism and teaching. He is the author of several books published in Spanish, including *The Origin of Life*, which has become a bestseller with over 650,000 copies sold. He is considered the foremost promoter of evolutionary biology and the study of the origins of life in Latin America, and has been Professor-in-Residence or Visiting Scientist in France, Spain, Italy, Cuba, Switzerland, Russia and the USA. He has organized a number of international symposia and scientific meetings, and has been member of number of editorial boards of major journals. He has received honorary degrees form the University of Milano (Italy), and the State University of Henan (China), the First Francesco Redi medal from the Italian Astrobiology Society, the Fundador Gold Medal from the Univeristy of San Francisco de Quito (Ecuador), and the Research Award in Natural Science from UNAM (2008), among others. Antonio Lazcano has served on many international advisory and review boards for NASA and other international organizations. He was twice President of the International Society for the Study of the Origin of Life, the first Latin American scientist to occupy this position.

NATURAL HISTORY, MICROBES AND SEQUENCES: BACK TO THE ORGANISM?

Identification of an organism implies the existence of a preconceived scheme of the nature of life and how it can be divided into groups, which may or may not be ordered in hierarchical schemes. As shown by many examples of the misidentification of fossil remains, the changing nature of our description of the biosphere can deeply affect our understanding of biological diversity and its development through time. Although it is now recognized that microbes (both prokaryotic and eukaryotic) represent the oldest, most diverse living group, their identification and study is still tainted by deeply rooted scientific prejudices that date back to the times in which a binary division of organisms into plants and animals was held, and by the commonly held mistaken view that they are, for the most part, harmful pathogens. The surprising development of molecular biology techniques (which now allow us to rapidly sequence the entire genome of an organism), combined with the availability of faster, less expensive computers, has opened new venues for the study of biodiversity, including barcoding. In spite of its many advantages, however, this approach cannot be easily applied to the identification of microbes, including the symbiotic populations without which no animal or plant group can survive. No kind of knowledge of the biosphere is more important than another, and a balanced view of life histories, morphology, physiology and the like is required –and should be taught in our schools, a conclusion which implies that the venerable tradition of natural history should be recovered and updated with the contemporary molecular approaches.

Plenary Session Speakers & Abstracts

Panel Discussion: Progress since Second International Conference
(Scott Miller, Moderator)

PAUL HEBERT

A native of Kingston (Ontario), Paul completed a BSc in biology at Queen's University, a PhD in genetics at Cambridge University and postdoctoral fellowships at the University of Sydney and the Natural History Museum (UK). He has held faculty positions at the University of Windsor and at the University of Guelph where he is now a Canada Research Chair in Molecular Biodiversity. Over his career, Paul has served as Director of the Great Lakes Institute in Windsor, as Chair of the Department of Zoology at Guelph, and of the Huntsman Marine Science Centre in St. Andrews. He is currently Director of the Biodiversity Institute of Ontario and of the Canadian Barcode of Life Network. He has published more than 300 papers, most employing molecular approaches to probe issues such as breeding system evolution, species boundaries and identification. He is a Fellow of the Royal Society of Canada and a member of the Expert Panel on Biodiversity Science established by the Council of Canadian Academies.

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iBOL - THE INTERNATIONAL BARCODE OF LIFE PROJECT

Paul D.N. Hebert

Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada

The International Barcode of Life Project has one overarching goal - to create a digital identification system for eukaryotes. iBOL began with a workshop in June 2007 that assembled delegates from 20 nations to gauge scientific interest in the project. Discussions were highly positive, and reinforced the conclusion that iBOL would only succeed through an alliance between nations with high biodiversity and those with the sequencing facilities and data centres needed to generate and protect the barcode records. However, iBOL needed more than enthusiasm and an overall research plan; it required funds to build scientific capacity in varied areas, to carry out the work and to develop administrative structures. There has now been substantial progress in gaining the support needed to meet these varied challenges. As a consequence, IBOL has an international Board of Directors and a Scientific Advisory Board. New core facilities for barcode analysis and new informatics capacity are in-place or under construction in six nations. While the presence of this infrastructure will be an important enabler, iBOL also needs \$150M in operating funds – for sequencing reagents, for laboratory staff and for collection programs. Although the acquisition of this support has proven complex, much progress has been made – enough to declare that iBOL will see formal activation in July 2010. Phase I of iBOL will generate barcode records for 5 million specimens by 2015. A decade later, the iBOL community should have completed a barcode library for all eukaryotic life – an exciting prospect.

MARIA PÉREZ ORTIZ

Dr. Tila María Pérez Ortiz is an Acarologist from the Universidad Nacional Autónoma de México (UNAM) where she has been the Director of the Instituto de Biologia since 2003. She is also the Curator of the National Collection of Mites. She obtained her B.S. degree, her M.S. and Ph.D. at the UNAM and she was a visiting faculty member at Harvard Museum of Comparative Zoology for two years (1989 – 1991).

Dr. Pérez was the President of the XI International Congress of Acarology held in Mérida, Yucatán in 2002 . She has published on the biology, taxonomy, and ecology of feather mites describing over one hundred new taxa.

She was the Coordinator of the Graduate Program of Biological Sciences at the UNAM. She was also the Director of the MAB Biosphere Reserve Chamela-Cuixmala in the State of Jalisco. She is now a member of the Academic-Technical Committee of the CONACYT BARCODE OF LIFE NETWORK for Mexico.

MEXBOL, THE MEXICAN BARCODE OF LIFE NETWORK

Tila María Pérez-Ortiz

Instituto de Biología, Universidad Nacional Autónoma de México, México, DF, México.

After two years of preparations, in late 2008 the Mexican agency devoted to the support of science and technology – CONACYT – crystalized support for 13 research networks, one of them the BARCODE OF LIFE for Mexico. Initially, three academic institutions from different parts of Mexico and one government agency showed strong interest in being part of this network. The three academic institutions were el Colegio de la Frontera Sur (ECOSUR), el Instituto de Biología, UNAM, and el Centro de Investigaciones Biológicas del Noroeste (CIBNOR). The goverment agency was la Comisión para el Conocimiento y Uso de la Biodiversidad (CONABIO). Several workshops and meetings have been organized across the country, mainly during 2009. Additionally in mid-2009, CONABIO issued a call for applications to support barcoding projects. Sixteen projects were approved, coming from researchers from seven institutions. Finally in the second half of 2009, CONACYT issued two important calls for applications as part of this project. One is for the research community to become part of the network. Sixty-two group leaders from twelve institutions applied, and their applications are under review. The second important call was to support labs or for specialized shared equipment. With these activities, the funding agencies are giving a strong impulse to DNA barcoding in Mexico. Some results from this support will be presented during this conference.

Plenary Session Speakers & Abstracts

PETE HOLLINGSWORTH

Pete Hollingsworth heads the Genetics and Conservation programme at the Royal Botanic Garden Edinburgh. His research uses genetic tools to study plant biodiversity. The aims of this research are to: (1) gain an increased understanding of the processes governing the evolution of plant populations and species, with a particular emphasis on those processes which lead to diversification and taxonomic complexity, (2) develop genetic approaches for characterising plant species diversity, such as DNA barcoding, and (3) provide guidelines and practical applications of genetic methodologies in plant conservation. He has a PhD in plant population genetics from the University of Leicester, and Honorary Fellowships from the University of Edinburgh, University of Glasgow, and the Scottish Crop Research Institute. He has published over 70 papers on molecular ecology and systematics, and co-edited the volumes "Molecular Systematics and Plant Evolution" and "Speciation in Plants and Animals: Pattern and Process". He currently chairs the Plant Working Group of CBOL, and the Plant Working Group of the iBOL project.

PLANT BARCODING

Peter M. Hollingsworth

Royal Botanic Garden Edinburgh, Edinburgh, UK

Globally, there are about 400K species of land-plant. The effective conservation, utilisation and management of this botanical biodiversity requires that these plant species can be delimited and identified. However, high levels of species-diversity, coupled with the difficulties of undertaking biological identifications on sterile or fragmentary materials, makes the task of characterising the plant diversity of a given region or taxonomic group challenging.

DNA barcoding has the potential to address this issue and to enhance the global capacity for undertaking plant identifications. As CO1 evolves too slowly to serve as a useful barcode for plants, the first step for the plant barcoding community has been to identify a suitable land plant barcode. Comparative evaluation of candidate barcoding loci has been undertaken and this has resulted in formal recommendations to CBOL on the standard land plant barcode. CBOLs decision, and the announcement of the 'standard' land plant barcode will be made in time for the 3rd International Barcode of Life Conference.

The formal agreement on a standard plant barcode will lead to synergies among projects, and will enable acceleration of plant barcoding initiatives. Two pressing challenges are (a) to develop laboratory protocols and bioinformatics tools to facilitate the effective and routine use of this plant barcode, and (b) to continue to explore supplementary approaches to enhance levels of species discrimination in situations where the standard plant barcode does not provide adequate resolution.

Plenary Session Speakers & Abstracts

Session 2: Planning Meso-American or New Regional Barcoding Activities: Experiences from 2004-2009

SARAH ADAMOWICZ

After completing her BSc at Dalhousie University, Sarah Adamowicz pursued her MSc at the University of Guelph with Paul Hebert and used genetic tools to study species diversity, species boundaries, and polyploidy in *Daphnia*. Supported by a Beit Fellowship and NSERC Scholarship, she completed her PhD with Andy Purvis at Imperial College London, where she explored macroevolutionary patterns of diversification in the Crustacea as well as long-term evolutionary trends in morphology. She then moved on to the University of Waterloo to hold an NSERC Post-doctoral Fellowship with Jonathan Witt. Their work together included comparative studies of diversification patterns in invertebrates inhabiting ancient lakes and a case study on the endemic amphipod radiation of Lake Titicaca. Sarah has recently joined the faculty at the University of Guelph and remains deeply interested in the diversity of life in all its facets ranging from the molecular to community levels. She contributes to the Polar Barcode of Life and Barcoding Biotas themes of iBOL, with a particular focus on co-ordinating a large-scale biodiversity investigation of a single sub-Arctic site (Churchill, Manitoba, Canada) using DNA barcoding methods. Further, Sarah is working towards developing these vast DNA sequence resources for broad exploratory and hypothesis-driven biodiversity science.

CHALLENGES AND STRATEGIES TO ORGANIZE AN ATBI CAMPAIGN

Sarah J. Adamowicz

A large-scale effort has been undertaken over the past four years to barcode the entire multicellular biota at one site in sub-arctic Canada, encompassing tundra, boreal forest, marine, and freshwater environments. This project has involved over 120 students and collaborating experts and has generated over 25,000 barcode records to date. One unique component of this project is the heavy involvement of 20 Arctic Ecology field course students per year. The students focus on their individual research projects during the second week, investigating the biodiversity, distribution patterns, or biological or environmental associations of a particular taxonomic group. They have the opportunity to prepare 1-2 plates of specimens for DNA barcoding. To my knowledge, this is the only field course that includes an extensive component of DNA barcoding. The sequences are run at the Canadian Centre for DNA Barcoding, and the students analyze their data using BOLD. This collaboration with the students has been highly fruitful, with many students feeling this was a very positive learning experience. One critical component is for follow-up field collections by experts in particular taxonomic groups. Expert collectors typically collect one-third to one-half more species than do the students. Additionally, excessive duplication of common species has been a common problem, but this has been reduced over time through improved education about natural abundance patterns. Identifications of barcoded specimens will take longer and is ongoing with the help of numerous collaborators. In addition to contributing preliminary surveys of various taxonomic groups, this approach has proven very positive for graduate student recruitment, leading to more complete surveys and interesting studies.

Plenary Session Speakers & Abstracts

KAREN JAMES

Karen James is a postdoctoral researcher in the Department of Botany at the Natural History Museum in London and a participant in CBOL's Plant Working Group. She carried out floristic DNA-barcoding as part of a project to repeat Darwin's 1855 botanical survey of Great Pucklands meadow near Down House in Kent, and is beginning a new project to barcode the British tree flora with the participation of primary schoolchildren and other non-experts. Karen also coordinates the museum's Darwin bicentenary science campaign including a survey of the museum¹s Darwin specimens and a Galapagos mockingbird conservation genetics project. In addition to DNA barcoding, Karen has published on genetics, developmental biology, biodiversity genomics and DNA-based species identification and is an active science blogger and twitterer. Karen is also the director of science for The HMS Beagle Trust which aims to build a modern seagoing version of HMS Beagle for scientific research, public engagement and learning.

NOT JUST END-USERS BUT PARTICIPANTS: EXPLORING THE INTEGRATION OF PRIMARY SCHOOL STUDENTS AND OTHER NON-EXPERTS INTO BARCODING PROJECTS

JAMES, K.E.

Natural History Museum, London, UK

A key justification for DNA barcoding is its potential to increase and improve public engagement with the natural world. CBOL's mission is not only to assemble barcode reference libraries, develop technology and encourage global participation of taxonomists, but also to promote 'the use of DNA barcoding for the benefit of science and society'. We have recently partnered with a UK educational charity to explore how schoolchildren may be engaged now in the generation – not just use – of DNA barcode reference databases. 'Project BarkCode' will DNA-barcode the British tree flora while piloting methods for involving children and other non-experts in DNA barcoding campaigns. While this project is still in its early stages, we are beginning to accumulate a list of 'lessons learned' and other resources including: 1) the story of our non-traditional funding approach (in our case a private educational charity rather than a a research council, the latter having been repeatedly and unsuccessfully targeted), 2) the results of our preliminary workshops with science teachers to ensure an optimal educational eperience while still producing high-quality data, 3) a proposed workflow to be tested in Spring 2010 and 4) plans and specifications for remodeling a classroom building at a newly acquired science education centre in Dorset as a self-contained herbarium and DNA barcoding facility.

MIKE WILKINSON

Plenary Session Speakers & Abstracts

ANDY POLASZEK

Andrew Polaszek is a Researcher in entomology at the Natural History Museum, London. He specialises in parasitoid wasps, especially chalcidoids, but also has an active interest in bees, ants and other Hymenoptera. He has participated in several classical biological control programmes with parasitoid wasps, including the successful introduction of two species into Trinidad against the citrus blackfly, a major pest.

Andrew completed his PhD at Imperial College, London, before joining the Commonwealth Institute of Entomology in 1985. From 1990-1994 he was Principal Investigator in the Department of Entomology at Wageningen Agricultural University (The Netherlands) studying cereal pests and their natural enemies in sub-Saharan Africa. In 2004 he was appointed Executive Secretary of the International Commission on Zoological Nomenclature (ICZN). At ICZN he was involved in the establishment of the ZooBank register of animal names and nomenclatural acts, and as a consequence maintains a strong interest in the development of the Code, especially in relation to electronic publication of systematic work, including web-based taxonomy. He has authored more than 100 scientific publications, edited the book "*Cereal stem borers in Africa*" (CABI Bioscience, 1998) and its French translation (CIRAD, 2000) and has co-edited *Journal of Natural History* since 1995.

FACILITATING FUTURE TAXONOMY

Andrew Polaszek

Natural History Museum, London, UK

Valid taxonomic work has always been published in static ink-on-paper journals or monographs. From monumental revisionary studies to minor nomenclatural acts, all taxonomy must be published in this way in order to be valid. The International Code of Zoological Nomenclature expressly excludes nomenclatural acts, including all taxon descriptions, that are published in an electronic-only form. This traditional approach has its basis in guaranteeing the permanence of the taxonomic archive.

However, scientific journals are increasingly electronic-only, and there are many good reasons for this transition, not least the conservation of our forests. At the same time, the dynamic and ever-changing character of systematics, with new hypotheses arising regularly, suggests that a dynamic forum for taxonomy is preferable to static ink-on-paper works that often have built-in obsolescence. The revolution in zootaxonomy that we are currently experiencing, that includes DNA data and unparalelled imaging and data storage capabilities, requires a dynamic medium for its publication. Web taxonomy is undoubtedly its future, and can be facilitated and safeguarded by global taxonomic community projects such as the EDIT scratchpads and the ZooBank register of taxon names and nomenclatural acts.

Plenary Session Speakers & Abstracts

VAZRICK NAZARI

Vazrick Nazari received his MSc in Systematics and Evolution from University of Alberta in April 2006, and joined the Hebert lab as a PhD candidate in September 2006. He is co-advised by Jean-Francois Landry (CNC, Ottawa) and Paul Hebert. His research involves testing the utility of DNA barcodes in determining species boundaries and rates of diversification among North American microlepidoptera.

For students of ecology or biology: DNA barcodes can be phenomenally useful in sorting out mixed samples collected during ecological and biodiversity studies. Barcoding can minimize the effort needed to identify every single individual in such mixed samples by sorting them into clusters, where a single individual from each cluster can then be sent to the respective taxonomist for identification. Barcoding can also help you to find out which male goes with which female – something even taxonomists cannot help you with in many cases.

For taxonomists: Barcoding has many useful properties: It can help you associate sexes as well as various life stages of a species; It can reveal hidden diversity or show where a single species has received multiple names in the past. However, difficult groups of lower taxa, traditionally identified as "species complexes", often involve geologically very young species that have diverged recently (<1 million years ago) and are still in the beginning of the differentiation process. If you are a taxonomist struggling with such a group, trying to figure out the number of species involved and their boundaries, it is important to remember that the mitochondrial DNA in this case has likely not accumulated enough base-pair differences needed to tell these entities apart, and so their DNA barcodes will not be very helpful for you either.

Plenary Session Speakers & Abstracts

DAN JANZEN
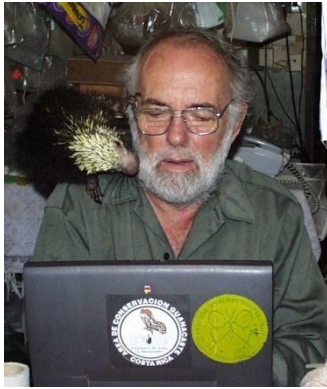
Daniel Janzen is DiMaura Professor of Conservation Biology, University of Pennsylvania, and Technical Advisor to Area de Conservacion Guanacaste (ACG), djanzen@sas.upenn.edu. ACG is a 163,000 hectare conservation area in northwestern Costa Rica. He is a tropical ecologist and biodiversity conservationist with 56 years of field experience and 444 scientific papers and books, all focused on the interactions of tropical animals and plants, and for the past 25 years, their permanent in-situ conservation as well. He is a member of the US and the Costa Rican National Academy of Sciences, and recipient of the Crafoord Prize and the Kyoto Prize. He and his biologist wife Dr. Winnie Hallwachs are co-architects and co-constructors, along with many others, of ACG and of Costa Rica's INBio (Instituto Nacional de Biodiversidad), and of Costa Rica's new Iniciativa Paz con la Naturaleza (IPN). He and Hallwachs are currently focused on facilitating the CBOL and iBOL efforts to DNA barcode the species of the world for their identification by anyone anywhere at any time, and simultaneously, on facilitating Costa Rica's willingness to permanently conserve the 4% of the world's biodiversity that lives on 25% of its national terrain.

PARATAXONOMISTS AND BARCODING IN TROPICAL BIODIVERSITY INVENTORY AND DISCOVERY

Dan Janzen (1,2)

(1) Area de Conservacion Guanacaste, Costa Rica
(2) University of Pennsylvania, Philadelphia, PA, USA

We currently lack the ability to massively barcode the world's museum collections. With much of tropical biodiversity not yet collected/inventoried anyway, and especially in conserved wildlands, a major step to building universal cross-taxa bioliteracy towards wildlands is massive and industrial scale inventory barcoding of select complex tropical sites. While fragments of this task can of course be carried out by undergraduates, graduate students, postdocs and Ph.D. researchers at a high time and dollar cost, major advances can also be made through a dedicated parataxonomist work force, drawn from the residents of the agroscape, "at home" in it, and integrated with the taxasphere. In addition to being technically and sociologically feasible while economical, the parataxonmist career provides entry-level intellectually-rich employment and share-holder by-in. And it certainly increases the spread of scientific and conservation understanding through gentle familiarity rather than forced education and legislative dictates. Parataxonomist on-the-job training and direct performance is elegant adult job creation within the working class as a key ingredient of conservation and bioliteracy through biodiversity development. And it works. Unfortunately, it also can be resisted by other social sectors not pleased by an increase in a competing work force.

Plenary Session Speakers & Abstracts

Session 3: Case Studies: Impact of barcode data in research areas beyond taxonomy

DARÍO LIJTMAER

Darío Lijtmaer is a researcher of the National Research Council of Argentina (CONICET), working in the Ornithology Division of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", where he is in charge of organizing and curating the bird frozen tissue collection. He holds a B.S. and a Ph.D. in Biological Sciences from the University of Buenos Aires, obtained in 2001 and 2008, respectively.

His research interests are related to diverse aspects of avian evolutionary biology, including 1) communication, 2) speciation, and 3) diversification patterns of Neotropical birds, studied through phylogenetic and phylogeographic approaches. His research in these areas is integrated with his involvement in the Barcode of Life project, more specifically in barcoding the birds of Argentina and other Neotropical countries.

BARCODING LARGE SETS OF BIRDS ALLOWS INSIGHTS INTO PATTERNS OF EVOLUTION, BIOGEOGRAPHY AND SPECIATION

Darío Lijtmaer(1) & Kevin Kerr (2)

(1) Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina.
(2) University of Guelph, Guelph, Ontario, Canada.

The causes of the increased bird diversity in the Neotropics as compared to the Nearctic have been a subject of much debate. The traditional view was that high tropical diversity is a consequence of high speciation rates, but it has been recently proposed that the reason is the presence of higher extinction rates in temperate regions. Also of interest is the comparison of processes that generate diversification and structuring in each region. Large sets of barcodes allow studying these continental patterns. We compare here the recently obtained barcodes of 500 bird species from the southern Neotropics (Argentina) with those of North America. This comparison shows that species of both regions have similar average intraspecific divergences in COI (2.4% in Argentina, 2.3% in North America). Average interspecific divergence between closely related species is slightly higher in Argentina (6.2% divergence between nearest congeneric neighbors versus 4.7% in North America). In addition, a lower proportion of closely related species show very low divergence in the southern Neotropics: 8% of nearest congeneric neighbors diverge by less than 1% in Argentina and 20% in North America. Finally, the proportion of species with genetic structure and high intraspecific variation is similar in both regions, but the patterns of geographic structure are more complex in Argentina. Taken together, these results indicate that there are not more young species in the southern Neotropics compared to the Nearctic, suggesting that speciation rates are not higher in the former. Instead, species appear to be slightly older in the southern Neotropics.

Plenary Session Speakers & Abstracts

C.J. GERACI

CJ Geraci, originally from New Jersey (USA), received a Master's in Ecology from the University of North Carolina at Chapel Hill and a PhD in Entomology from Clemson University. Her research interests include taxonomy and systematics of caddisflies (Trichoptera) and tumbling flower beetles (Coleoptera: Mordellidae), biodiversity of rainforest canopies, and aquatic ecology. She has worked with colleagues from the Trichoptera Barcode of Life Campaign since 2007 to barcode the NMNH caddisfly collection in support of bioassessment and biomonitoring. Currently, she is working with Nature Iraq to produce a DNA barcode library and key to the aquatic insects of Kurdistan and Iraq's southern marshes. Her postdoctoral research at NMNH is with Terry Erwin on the Ecuador Canopy Biodiversity Project, which is a combined effort to monitor the impacts of road construction and oil extraction on biodiversity near Yasuni National Park. Recently, she has been working with Dave Erickson and John Kress to develop protocols for documenting insect feeding via DNA barcoding on Plummer's Island, MD (USA).

DNA BARCODING AND ECOLOGICAL FORENSICS: HOW WE CAN USE THEM TO STUDY INSECT-PLANT RELATIONSHIPS

C.J. Geraci, D. Erickson, W.J. Kress , & T.L. Erwin

National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

As reference libraries are expanded to include plants, DNA barcoding becomes a powerful tool for testing ecological hypotheses of insect feeding strategies. In this study, we used DNA barcoding as a forensic tool to survey the feeding specificity of a wide array of insect taxa collected on Plummers Island, Maryland, USA. Plummers Island (PI) has been studied by field biologists for over a century. Its flora and fauna are well known and, most importantly, all resident plant species have been DNA barcoded. We used multiple sets of primers to sequence the mtCOI barcode region from the insect body tissue and the coding regions rbcL AND matK together with the trnH-psbA intergenic spacer region from the gut contents of a wide variety of insects. We then used the PI DNA barcode library to identify the plant species present in those gut contents. So far our survey has found plant DNA from at least four plant families in the guts of individuals from the Orders Coleoptera (Carabidae, Chrysomelidae, and Curculionidae), Hemiptera-Homoptera (Aphididae), and Lepidoptera. We also found evidence of plant feeding in beetles previously classified as predators and scavengers, and also of polyphagy. In this talk we will summarize the results of the ongoing PI survey, describe methodological challenges for gut content barcoding, and explore the potential for using DNA barcoding to compare insect feeding strategies across the 50 hectare forest plots managed by the Center for Tropical Forest Science and the Smithsonian Institution Global Earth Observatories (SIGEO).

Plenary Session Speakers & Abstracts

ALEX SMITH

Alex is an Assistant Professor at the Biodiversity Institute of Ontario and the Department of Integrative Biology, University of Guelph, Ontario, Canada. His research involves both molecular and field ecological approaches as they pertain to questions of species evolution, population connectivity, and biodiversity. After receiving his PhD from McGill University in 2004, Alex took up a Fonds québécois de la recherche sur la nature et les technologies B-3 postdoctoral research fellowship to study species discovery and diversity assessment using DNA barcodes. Following the completion of this fellowship, Alex worked for two years as a research associate helping to establish the Biodiversity Institute of Ontario. Alex was recently hired as an Assistant Professor of Molecular Ecology on 100% secondment to the Biodiversity Institute. Alex works in close collaboration with Daniel H. Janzen and Winnie Hallwachs on parasitoid insects in the ACG of Costa Rica, with Brian L. Fisher on the ant fauna of the Malagasy region, and with amphibians and various insect groups on numerous projects ranging from phylo- and biogeography to species discovery, biodiversity assessment and community ecology.

INTEGRATING COMMUNITY ECOLOGY AND DNA BARCODING

Alex Smith (1), K.S. McCann(2), & E.S. Eveleigh(3)

(1) Biodiversity Institute & Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada.
(2) Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada.
(3) Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, New Brunswick, Canada

Throughout eastern North America, the population dynamics of spruce budworm (Choristoneura fumiferana) are governed by insect parasitoids, (wasps and flies) operating at three trophic levels. These parasitoids are critical components of this complex food-web that canalize energy and nutrient flow dependent on whether they attack many hosts (a generalist) or a small number (or single) species (a specialist) in the ecosystem. Currently, the majority of the parasitoids in the spruce budworm food-web are considered to be generalists. We barcoded the spruce budworm food-web to test whether the presumably polyphagous parasitoids are, in fact, morphologically-cryptic host-specialists. We added a nuclear marker to this barcode library to test how phylogenetic community modeling of a parasitoid's host-specificity and position in the food-web affected energy and nutrient flow predictions.

Food-web barcoding provides an effective tool for the accurate identification of those species involved in the cascading effects of future budworm outbreaks. However, the more fundamental facet of how this diversity is actually connected on the landscape remains to be addressed by resource managers and ecologists. It is these connections, not the diversity itself, which ultimately dictate ecosystem function (e.g., herbivory, parasitism rates). Barcoding the budworm food-web delineated specific interactions with unparalleled precision and speed while phylogenetic analyses of trophic connectivity provided more accurate community ecological models. Integrating phylogenetic estimates of standardized barcodes permits ecologists and resource scientists to move from a strictly population-based approach to a more unified conceptual basis (i.e., from population to food-web). We feel that integrating barcoding techniques with food-web ecology will change the face of community ecology.

Plenary Session Speakers & Abstracts

ESKE WILLERSLEV

Eske Willerslev, an internationally recognised researcher in the fields of ancient DNA, DNA degradation, and evolutionary biology, is director for Centre of Excellence in Geogenetics, situated at the National History Museum and the Biological Institute, University of Copenhagen. During his PhD he established the first ancient DNA facility in Denmark, which, despite its small size, rapidly became internationally recognized for, among other things, establishing the fields of ancient sedimentary and ice core genetics, which have since become world-wide scientific disciplines. After finishing his PhD studentship he obtained a prestigious Wellcome Trust Fellowship to join the Department of Zoology at the University of Oxford, UK – a world-leading institution in many fields of research, including ancient DNA. Recently, at the age of 33, he was called back to University of Copenhagen to commence the position of Full Professor, first at the Niels Bohr Institute and later at the National History Museum and Biological Institute. In addition, he has been awarded the prestigious position of Visiting Professor by Oxford University.

His research interests include: palaeoecology, palaeontology, archaeology, domestication, ancient microbial biology, phylogenetics, molecular evolution and barcoding. He has published repeatedly in such prestigious journals as Science and Nature, and has served as a reviewer for various grant agencies and journals. He has strong collaborations with world leading scientists in Europe, USA, Canada, and Russia, and participated in 12 international polar expeditions, 5 of which he led. He has communicated his work to the public through documentary films, books, popular articles, museum exhibitions and numerous national and international TV, newspaper, and magazine interviews.

HUNTING THE MOLECULAR PAST

Eske Willerslev

University of Copenhagen

In the past two decades, ancient DNA research has progressed from the retrieval of small fragments of mitochondrial DNA from a few late Holocene specimens, to large-scale studies of ancient populations, phenotypically important nuclear loci, and even genome scale sequencing of extinct species. However, the field is still regularly marred by erroneous reports, which underestimate the extent of contamination within laboratories and samples themselves. An improved understanding of these processes and the effects of damage on ancient DNA templates has started to provide a more robust basis for research. Recent methodological advances have included the characterization of Pleistocene mammal populations and discoveries of plant, insect, and vertebrate ancient DNA preserved in ancient hair, coprolites, sediments, and ice using DNA barcoding approaches. These have changed our understanding of human migration into the New World and the palaeoecology of Greenland, Alaska, and Siberia. Increasingly, ancient genetic information is providing a unique means to test assumptions used in evolutionary and population genetics studies to reconstruct the past. With the advent and uptake of appropriate methodologies, ancient DNA is now positioned to become a powerful tool in biological research.

Plenary Session Speakers & Abstracts

Session 4: Informatics and Data Analysis

NEIL SARKAR

Indra Neil Sarkar, PhD, MLIS, is the Chair of the Data Analysis Working Group for the Consortium for the Barcode of Life. He is the founding director of biomedical informatics at the University of Vermont within the Center of Clinical and Translational Science. He has faculty appointments in both the Department of Microbiology & Molecular Genetics and the Department of Computer Science at the University of Vermont. Prior to his current positions, he held scientific appointments at the American Museum of Natural History (New York, NY) and the Marine Biological Laboratory (Woods Hole, MA). His specific research involves the development and use of a range of computational techniques (including knowledge gathering and discovery methods, phylogenetics, information theory, ontology development, semantic indexing, and natural language processing) in the study of disease. Dr. Sarkar's work ultimately aims to integrate biomedical and biodiversity knowledge to facilitate the generation of comparative biology hypotheses that can be ultimately tested within a translational science framework. Dr. Sarkar's background in microbiology, melded with formal training in biomedical informatics and library science as well as his research applications in healthcare, provide him with a unique bridging perspective across a wide range of biomedical domains. His current research is funded by the National Institutes of Health and the National Science Foundation.

BARCODE ANALYTICS: WHEREFROM, WHERENOW, & WHERETO?

Neil Sarkar

University of Vermont
Chair, Data Analysis Working Group, Consortium for the Barcode of Life

From the early days of DNA Barcoding, a dizzying array of possible informatics approaches have been discussed for the analysis and ultimate use of DNA barcode data. There are numerous arguments for or against any given method (i.e., phenetic, cladistic, statistic, stochastic, etc.). One of the core missions of the Consortium for the Barcode of Life's Data Analysis Working Group (DAWG) has been to develop an objective paradigm for proposing and evaluating the range of possible approaches for analysis of barcode data.

This presentation will begin with a discussion of how DAWG has been able to successfully address this charge to date, culminating with a presentation of the Barcode of Life Data Portal (BOLI-DP; http://boli.uvm.edu). The discussion will conclude with a look to the future of the informatics and data analysis within the scope of DNA barcoding under the purview of DAWG, with a particular emphasis on data visualization in practical contexts.

Plenary Session Speakers & Abstracts

SUJEEVAN RATNASINGHAM

Sujeevan Ratnasingham is the Informatics Director at the Biodiversity Institute of Ontario, University of Guelph and the Chief Architect of the Barcode of Life Data System (BOLD). He has a background in Computer Science from the University of Guelph with a focus on high performance computing and database analysis. Lately, his research has focused on machine learning approaches to sequence analysis and unsupervised clustering. He joined the Hebert lab in 2003 as one of the first researchers focused on DNA barcoding, providing bioinformatics support in the assessment of single gene markers to delineate animal species. His contribution to DNA barcoding has continued since then with the development and expansion of BOLD, development of high-throughput barcoding methods, and aiding in the establishment and implementation standard DNA barcodes for plants. As a member of the CBOL Database Working Group and CBOL Implementation Board, he has been heavily involved with the establishment of data standards and expansion of the barcoding community.

MULTI-LOCUS SUPPORT AND BARCODE CLUSTERS IN THE BARCODE OF LIFE DATA SYSTEM (BOLD)

Sujeevan Ratnasingham

Biodiversity Institute of Ontario, University of Guelph, Guelph, ON N1G 2W1, Canada

The Barcode of Life Data System (BOLD), activated in 2005, has become the primary workbench for the assembly, analysis, and publication of barcode datasets. Until recently, the focus of development has been on supporting animal barcoding. The maturation of plant, fungal, and protist barcoding required new capabilities, most importantly, the capacity to store multi-locus barcodes. New analytical tools and management functionality have also been incorporated to help manage and visualize complex data sets, especially those in multi-locus projects. An important development has been the implementation of the Barcode Index Number system (BIN). This system consists of two parts 1) a novel clustering algorithm employing graph methods to generate operational taxonomic units (OTUs) and putative species from sequence data without prior taxonomic information, and 2) a curated registry of barcode clusters integrated with an online database of specimen and taxonomic data with support for community annotations. The BIN framework can greatly expedite the evaluation and annotation of described species and putative new ones while reducing the need to generate interim names, a non-trivial issue in barcoding datasets. For example, nearly half of the species names on BOLD are interim and their number is growing at a rapid rate. The BIN algorithm has been effectively tested on a broad set of taxonomic groups and shows potential for applications in species abundance studies and environmental barcoding. The registry employs modern GUID and web service functionality enabling integration with other databases. A partnership with GenBank has led to handshaking of terms and identifiers with the BIN registry; other taxonomic and biological databases are being pursued for collaboration.

Plenary Session Speakers & Abstracts

JOAQUÍN GIMÉNEZ

Joaquín Giménez has a bachelor's degree in Biology from the National University of Mexico (Universidad Nacional Autónoma de México - UNAM). After graduating he worked for four years at the department of Digital Publications within the Computing Services Unit (DGSCA) of the same university. He specialized in the management of digital academic contents, electronic publishing standards and communication protocols.

In 2004 he founded and has since then coordinated, the Biodiversity Informatics Unit (UNIBIO) of the Biology Institute of UNAM. The UNIBIO is responsible for systematizing and publishing online all the available biodiversity information from the institute's scientific collections. Moreover, the UNIBIO also designs and implements systems to access and analyze this data.

He has participated in several large scale university projects to develop digital information systems such as SIBA (Sistema de Informática para la Biodiversidad y el Ambiente) that integrates biodiversity archives and geospatial data, and MUTIC (Macroproyecto de la Universidad de la Tecnología y la Información) to develop tools to access and manage academic information online and 3Rs (Red de Repositorios Universitarios) to form a network of institutional repositories connected through OAI.

Finally, Joaquin is also a member of the Center of Complex Sciences (Centro de Ciencias de la Complejidad), where he has lately worked on the use of complex networks and data mining for the analysis of emergent diseases and other ecological events.

INTEGRATING AND SHARING BIODIVERSITY INFORMATION USING WEB SERVICES ARCHITECTURE

Joaquín Giménez & R. Saenz

Universidad Nacional Autónoma de México

At present, Scientific museums should manage data bases, data entry and data search systems, and data analyses tools, besides satisfying all local investigators and curators needs, maintaining their inter-operability with diverse external initiatives such as BOL. An effective way to do that is with the implementation of Services Oriented Architecture (SOA), and following international standards and protocols such as Darwin Core for collections data, Dublin Core for digital objects and GML for Geographic's objects.

At UNIBIO-UNAM, we are developing a SOA implementation for the biological collections of the university. We built a data framework using PostgreSQL and PostGIS data bases to store and manage biological and geographical information, and Institutional Repositories to manage digital objects (images and papers) through OAI Protocol. The connection between portals and data frameworks is achieved through Web Services, which are able to interoperate different systems running on heterogeneous platforms in a transparent way. Web services have the technology to allow data exchange through standard formats (XML & JSON) and also support the communication in proved scalability protocols such as HTTP. The architecture has a layer of administration and query services, composed by a set of developed modular portals, developed with Ajax. This layer is connected to the Web services to access data, integrating the information of the biological collections with maps and digital objects (images). This integration is achieved using a shared code in the form of a triplet, which consists in the integration of InstitutionCode, CollectionCode and CatalogueNumber fields from the Darwin Core standard.

Finally there is another layer of Web Services that allows exporting the records selected by the user through the queries to portals to a new layer in which diverse tools of analysis and visualization are implemented.

Plenary Session Speakers & Abstracts

KASPER MUNCH

Kasper Munch is currently at the Bioinformatics Research Center at Aarhus University in Denmark, where he holds a temporary research position. He has a Ph.D. in computational molecular genetics from University of Copenhagen, Denmark and has previously worked on eukaryotic gene prediction and expression analysis.

As postdoc at Institute of Biology, University of Copenhagen he worked on statistical and computational aspects of genetic barcoding. This line of research has focused on devising robust and automated procedures that return a statistical measures of confidence in individual assignments. The Statistical Assignment Package (SAP) implementing these methods has formed through collaborations with experimental groups analyzing environmental ancient DNA to address extinction times and composition of ancient ecotypes. He recently ended a postdoc at Center for Theoretical Evolutionary Genomics, University of California, Berkeley where his research also included analysis of population structure in using ancient DNA.

His current interests in genetic barcoding include development of methods that deal with the fact that reference databases are often lacking in species coverage so that the unknown species may in fact not be represented in the database. He is also interested in developing methods sufficiently fast to allow analysis of large next generation sequencing data sets.

STATISTICAL DISCOVERY OF UNSAMPLED POPULATIONS OR SPECIES

Kasper Munch (1), S.C. Choi (2), R. Nielsen (3) & J. Hey (2)

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Genetic barcoding relies on the assumption that the unknown species from which a sample sequence is obtained is represented in the reference database. Considering that the number of species lies somewhere between 2 and 100 million and that only about 85 thousand species have been barcoded so far, it is not likely that the barcoding databases will be complete any time soon. This means there is an unknown risk that the sample sequence is assigned to a closely related, but incorrect, species only because the correct species is not represented in the database. We propose a statistical approach to address this problem

Using a phylogenetic approach, the sample sequence is normally assigned to a species if the probability that it groups monophyletically with database sequences from this species is high. If the sample sequence preferentially falls as an outgroup to the sequences from that species, however, it is possible that it truly belongs to an unsampled sister species or isolated population not represented in the database. To this end we apply a Bayesian population genetic approach to calculate the posterior probability that the sample sequence does indeed come from such an unsampled population or species. This will allow researchers to quantify the risk of false assignment due to insufficient database coverage.

For genera where database coverage is regarded as exhaustive a high probability of assignment to an unsampled population may serve as independent initial evidence in the discovery of isolated populations or new species
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Plenary Session Speakers & Abstracts

Session 5: Case studies of Applications

PHAEDRA DOUKAKIS

Phaedra Doukakis, Ph.D., is a biologist interested in conservation of fishes and their environments. Her research interests include fisheries and marine protected area management and using genetic and field-based tools to study species diversity, evolutionary history, and population structure within an applied conservation context. Currently a Senior **Research** Scientist at the Institute for Ocean Conservation Science at Stony Brook University, her primary focus is the study of global sturgeon fisheries and the international caviar trade. Her work is of direct relevance to policy, specifically that set by the Convention on International Trade in Endangered Species (CITES). Dr. Doukakis has held positions at the Pew Institute for Ocean Science at the University of Miami, the American Museum of Natural History and the Wildlife Conservation Society. She holds a Ph.D. Ecology and Evolutionary Biology and M.S. and M. Phil. in Biology from Yale University where she studied sturgeon conservation biology and genetics with an emphasis on Eurasian species and the caviar trade. She received a BS in Biology from the University of North Carolina at Wilmington in 1994.

DNA-BARCODING OF SHARKS: TWO CASE STUDY APPLICATIONS IN BELIZE AND MADAGASCAR

Phaedra Doukakis. (1), D. Chapman(1), R. Hanner(2), M. Shivji, (3), C. Bartholomew (4), M. Benavides (1)& G. Amato (4)

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(2) University of Guelph, Guelph, ON, Canada
(3) Nova Southeastern University, Dania Beach FL, USA
(4) American Museum of Natural History, New York, NY USA

DNA barcoding can provide a quick and reliable method for characterizing the species composition of ecosystems and markets. We used DNA barcoding to improve management of sharks, which are exploited and threatened globally to support a lucrative international trade in their fins.

Antongil Bay in Northeastern Madagascar is home to important, yet unstudied, shark fisheries. A cooperative program was established with fishers to collect genetic samples and DNA barcoding was used to identify the species captured, use of the Bay as a breeding area, and partitioning of species and life stage amongst different fisheries. At least 17 species are harvested and six species utilize the Bay for seasonal reproduction with differences among fisheries in life stage and species. The study provides guidance for management and a baseline for future monitoring.

The Mesoamerican Barrier Reef (MABR)) is the world's second largest barrier reef. Considerable shark fisheries occur in the Belizean part of the MABR. We obtained the whole anal fin (a fin not used commercially) from every shark landed over a two year period. DNA-barcoding was used to identify the species origin of fins and test whether morphological determination of species identity is feasible from just anal fins. Our investigation provides an efficient way to monitor shark landings in Belize.

These two cases illustrate the vast amount of data that can be collected using DNA barcoding approaches. The cooperative approach further established contact with the user group ultimately involved in the application of the findings.

Plenary Session Speakers & Abstracts

GEORGE AMATO

Dr. George Amato is the Director of the Sackler Institute of Comparative Genomics at the American Museum of Natural History. In addition to administering this interdepartmental scientific program of more than 70 scientists, postdoctoral fellows and graduate students, Dr. Amato continues to conduct research in conservation genetics of endangered species. He also serves as an Affiliated Professor in the Richard Gilder Graduate School and is an adjunct professor at Columbia, Yale, and Fordham Universities. Previous to joining the Museum, Dr. Amato spent seventeen years conducting conservation research and programs at the Wildlife Conservation Society, where he was the Director of Conservation and Science until 2005. Dr. Amato has lectured and published extensively on conservation strategies for endangered species, concentrating much of his work on the use of molecular analysis to determine conservation priorities and in developing forensic tools for monitoring the illegal trade in wildlife. Additionally, he is the chairman of the Consortium for the Barcode of Life Conservation Committee and is also a Trustee of the Lemur Conservation Foundation and Rare Species Conservatory Foundation. Dr. Amato is involved in conservation issues on a global scale working on projects in Africa, Southeast Asia, and the Caribbean. He received his B.S. from the University of Connecticut and M.S., M.Phil. and Ph.D. from Yale University.

DNA BARCODING INITIATIVE FOR CONSERVATION (DBIC)

George Amato, Kolokotronis, S.O., Eaton, M.J., Naro-Maciel, E., Leslie, M. and Lowenstein, J.H.

American Museum of Natural History, New York, New York, USA

DNA barcoding methods have wide-ranging applications to help protect biodiversity against anthropogenic changes in the environment and the pervasive illegal commercial trade in animals and animal products. Endangered species that could benefit from a DNA barcoding approach include commercially hunted wildlife, wildlife consumed for the traditional medicine trade, rare species collected for private living collections, and unsustainable harvesting for other wildlife products. We have begun a genetic barcoding initiative targeting endangered species across the biodiversity-rich tropical forests and marine and freshwater environments of Asia, Africa, and Central and South America. Presented here are the results of three recent projects within this initiative. These include a study on wildlife bushmeat trade and crocodilian harvesting for twenty-three species from five vertebrate families (*Crocodilidae*, *Alligatoridae*, *Bovidae*, *Suidae* and *Cercopithecidae*). In this study multiple individuals were barcoded to estimate intraspecific sequence variability and document fixed diagnostic characters for species identification. Both fixed character differences and tree-based maximum likelihood distance methods unambiguously identified unknown and misidentified samples. In a second study on marine turtles, barcodes proved useful in identifying population structure for species that are threatened by high rates of fisheries bycatch. Finally, a third project focused on the commercial trade in Tuna sushi (genus *Thunnus*) demonstrates the utility of this approach for this over-harvested group.

Plenary Session Speakers & Abstracts

DANIEL MASIGA

Dan Masiga is a senior scientist at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya. He also holds a lectureship at the Department of Biochemistry and Biotechnology, Kenyatta University. Dan's main research focus is on arthropod borne infectious diseases. Having graduated with a PhD in molecular parasitology at Bristol University, he did a postdoctoral fellowship in the genetics of African trypanosomes at Glasgow University before returning to Kenya. His interest in barcoding is to promote the understanding to the diversity of arthropod vectors of infectious diseases in Africa, and the pathogens they transmit. This can help us understand better the epidemiology of infectious diseases, and more rationally define strategies for control. His laboratory is also involved in barcoding moths and fruit fly species. Dan is a member of CBOL's Implementation Board, and the Kenyan node of iBOL.

ANALYSIS OF MITOCHONDRIAL CYTOCHROME REVEALS KEY SOURCES OF TSETSE FLY BLOOD MEALS IN EAST AFRICA

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(2) Kenya Agricultural Research Institute, Trypanosomiasis Research Centre, P.O Box 362-00902, Kikuyu, Kenya;
(3) Tsetse & Trypanosomiasis Research Institute, P.O. Box 1026, Tanga, Tanzânia;
(4) Makerere University, Uganda.

Information on the vertebrate hosts of bloodfeeding vectors forms a useful decision-making tool in the planning control and eradication operations. The objective of this study was to investigate the utility of mitochondrial Cytochrome c oxidase I (COI) and Cytochrome b (Cytb) genes for identifying tsetse fly blood meals in order to provide a basis for more rational control of trypanosomiasis in East Africa. Glossina swynnertoni and G. pallidipes were sampled from the Serengeti (Tanzania), Nguruman and Busia (Kenya), respectively. Tsetse blood meal analysis was carried out by amplification and sequencing, to identify polymorphisms in the partial COI and Cytb genes with diagnostic value. Sequences obtained were used to query BOLD using the barcode of life data systems identification engine (for COI) (http://www.barcodinglife.org/views/idrequest.php) and the non-redundant nucleotide database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and hosts identified on the basis of percent identities. An initial assay showed that most feeds were from single sources. In the Serengeti ecosystem (Tanzania), hosts included buffalo (61%), giraffe (22%), warthogs (7%) and spotted hyena (2%). In Nguruman (Kenya) G. pallidipes flies fed on elephants (46%), warthogs (38%), while buffalo and baboons accounted for 8% each. Only cattle blood was detected in flies caught in Busia in western Kenya. Control strategies will be informed by the sources of bloodmeals. These results will also influence the direction in the search for chemicals that lure tsetse to different hosts, to enhance the efficacy of trapping technologies.

Plenary Session Speakers & Abstracts

JEREMY DEWAARD

Mr. deWaard is a PhD candidate in the Forest Sciences Department of the University of British Columbia in Vancouver, Canada and research associate at the Royal British Columbia Museum in Victoria, Canada. Prior to commencing his current degree, Jeremy completed his MSc in the laboratory of Dr. Paul Hebert at the University of Guelph in Guelph, Canada exploring the molecular evolution of animal lifestyle transitions. He remained at the Biodiversity Institute of Ontario for two years as laboratory manager, where he helped facilitate the transition to a high-throughput DNA barcoding facility. Currently, his PhD research focuses on genetics-based approaches to invasive species detection and surveillance, biodiversity monitoring and conservation prioritization. In particular, his work concentrates on developing regional barcode libraries for forest Lepidoptera, employing them to develop native and non-indigenous species inventories, and comparing diversity estimates across disturbance gradients.

FOREST BIOMONITORING, BIOSECURITY AND DNA BARCODING

Jeremy R. deWaard (1,2) & L.M. Humble (1,3)

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(2) Royal British Columbia Museum, Entomology, Victoria, British Columbia, Canada
(3) Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, British Columbia, Canada

The economic, sociological and biological value of our forests makes their sustainability essential to our well-being. To ensure their long-term health, it is critical to regularly and effectively monitor insect pests, as well as to detect introduced species early and accurately. These programs rely on the precise diagnosis of species, which can be complicated by sizeable trap samples, damaged specimens, immature life stages and incomplete taxonomy. The recent advent of DNA barcoding shows promise to circumvent some of the obstacles of pest monitoring and surveillance. We review several projects employing this tool for forest defoliators and other Lepidoptera in British Columbia, Canada. Firstly, we describe the integration of DNA barcoding into monitoring and detection programs, as set out by the International Standards for Phytosanitary Measures (ISPM). We highlight the utility of COI for detection with examples in tussock moths (*Lymantria* spp.) and the Poplar shoot borer (*Gypsonoma aceriana*). Secondly, we present the results of a faunal survey of nocturnal Lepidoptera in one of North America's largest urban parks. We reveal how DNA barcoding facilitated the inventory by minimizing specialist time and providing increased sensitivity of detection of species at low density, including four newly introduced species. And lastly, we discuss a study employing DNA barcoding to facilitate assessments of lepidopteran diversity across several disturbance gradients. We underscore the improvements that this approach provides relative to traditional techniques, such as allowing the appraisal of multiple levels of diversity—species, genetic and phylogenetic.

Plenary Session Speakers & Abstracts

BERNARD SWEENEY

Bernard W. Sweeney is presently Director, President, and Senior Research Scientist at the Stroud Water Research Center, an independent research institution focused on stream and river ecology located in Pennsylvania. He is also Vice-President of the Asociacion Centro de Investigacion Stroud, a non-profit Costa Rican corporation established to facilitate research and educational programs related to tropical stream ecology. He has an adjunct Professor appointment at the University of Pennsylvania. His research interests include the ecology and biodiversity of stream invertebrates, the role of streamside forests in the structure and function of streams, the genetic structure and secondary production of aquatic insects, stream pollution assessment, and stream restoration. He received the 2003 "National Award of Excellence in Conservation" from the USDA Natural Resource Conservation Service for his research on the restoration of streams and riparian corridors. In 2006, he received the "Lifetime Achievement Award" from the Chesapeake Bay Foundation and the "Margaret Douglas National Medal" from the Garden Club of America for work in conservation education. He is past president of the North American Benthological Society (NABS) and currently Co-Chairs the society's Taxonomic Certification Program. He will receive the Distinguished Service Award from NABS in 2010. He was appointed in 2008 to co-lead the Freshwater section of the International DNA Barcode for Life project.

WATER QUALITY ANALYSIS WITH MACROINVERTEBRATE BARCODING

Bernard Sweeney (1), T. Dapkey (2), J. Jackson (1), D. Funk (1) & J. Battle (1)

(1) Stroud Water Research Center, Avondale, Pennsylvania, USA;
(2) University of Pennsylvania, Philadelphia, Pennsylvania, USA

Macroinvertebrates identified three ways [student (amateur), professional entomologists, and DNA barcoding (cox1 gene)] were used to assess water quality (WQ) in White Clay Creek (WCC). We believe that the barcode data provide the first species level assessment of macroinvertebrate community structure and water quality in any stream in the world. Macroinvertebrates were collected quantitatively in WCC from an upstream station with good WQ (St11, score=14 of 20) and a station 2.8 km downstream with fair WQ (St12, score =7 of 20) due to landuse impacts. Most of the 1786 specimens involved were successfully barcoded (including early instars). A 2% genetic divergence provided good separation of presumptive species for all groups except Chironomidae Diptera (where 5% was used). The number of taxa distinguished by barcoding at the two sites (155) was significantly higher than estimated by the professional (96) or amateur (33). Each riffle contained a large number of species but many were extremely rare (48% with < 2 individuals). The species composition of the two stations differed significantly. The good WQ site (St11) had 40 species not found downstream at St 12 that, in turn, had 67 species unique to it. Of the 15 most common species in the study, only four occurred at each site with the rest being more or less unique to each site. The downstream degraded site had fewer pollution sensitive species than upstream (8 vs 11 mayflies, 2 vs 4 stoneflies, 7 vs 11 caddisflies) and significantly more species in pollution tolerant groups (71 vs 45 species of chironomids). Regardless, WQ scores based on professional and barcode data were identical using the "standard approach and metrics" and only slightly better than WQ scores based on amateur data. This failure to take full advantage of species level data reflects the lack of pollution tolerance information at the species level (especially for Chironomids), the use of bootstrapping to standard 150 individual counts (loss of information for rare species), and an emphasis on relative abundance of Order or Family level data in most water quality metrics. The study shows the utility of barcode data in gauging water quality from macroinvertebrate community structure as well as the need for new protocols, ancillary information, and metrics to take full advantage of species level identifications.

Plenary Session Speakers & Abstracts

Session 6: Barcoding and New Sequencing Technologies

MEHRDAD HAJIBABAEI

Dr. Mehrdad Hajibabaei is an Assistant Professor at Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, and Director of Technology Development for International Barcode of Life (iBOL) project.

Dr. Hajibabaei received his PhD in Biology (molecular evolution and bioinformatics) from the University of Ottawa in 2003. He then joined Dr. Paul Hebert's lab at Guelph, as the first postdoctoral fellow on DNA barcoding. During his postdoctoral research he pioneered the development of high throughput DNA barcoding and worked on DNA barcoding tropical Lepidoptera as well as barcode recovery from archival specimens. Dr. Hajibabaei is a founding member of the Canadian Centre for DNA Barcoding, where he continues his research on various aspects of DNA barcoding and molecular biodiversity from theoretical work to technology development and bioinformatics.

DNA BARCODING 2.0: ENVIRONMENTAL MONITORING USING NEXT-GENERATION SEQUENCING TECHNOLOGIES

Mehrdad Hajibabaei

Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

The Barcode of Life Initiative is building a standard reference DNA barcode library for all species. Barcode sequences have mainly been produced one sample at a time by using automated Sanger capillary sequencers. Although this workflow can be adopted for high-throughput analysis and a single facility can process several thousands of samples annually, the Sanger sequencing workflow is not feasible for the analysis of bulk environmental samples containing mixtures of species. For example, specimens used for standard biomonitoring applications contain a mixture of taxa often from immature stages or very small organisms. It is very difficult or sometimes impossible to sort and process these samples using standard barcoding workflow. Recently-introduced next-generation sequencing platforms provide the capacity to sequence thousands of target DNA templates in parallel; hence, these platforms have been applied in biodiversity analysis mainly for analysis of ribosomal sequences in microbial samples. We have established an analytical facility to utilize the Roche 454 sequencing platform for DNA barcoding environmental samples. We targeted the CO1 barcode region in mixed specimens of tropical Lepidoptera, fish, and arrays of macroinvertebrates for optimizing the approach and developing protocols. We have also developed a customized bioinformatics analysis pipeline to compare sequences with reference libraries and to visualize the results. Using this environmental barcoding approach we have been able to demonstrate differences in macroinvertabrate larval community structure in two river systems from an urban area and an adjacent conservation area near Guelph, Ontario, Canada. In addition to benthic samples, we have analyzed soil and water samples from these two sites targeting multiple markers for an assessment of total biodiversity using a phylogenetic profiling approach. Our results clearly demonstrate substantial differences in biota between the urban site and the conservation area, and these differences are reflected in several different taxonomic groups. Next-generation sequencers provide unprecedented power for generating sequence information and can therefore play a key role in understanding biodiversity through the analysis of DNA barcodes.

Plenary Session Speakers & Abstracts

THOMAS BRUNS
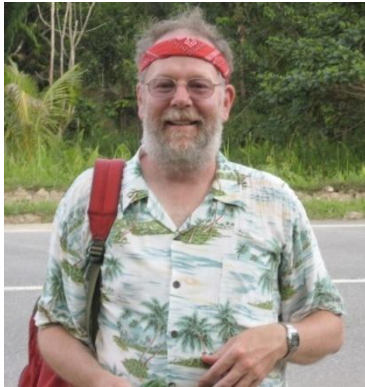
Tom Bruns studies fungal ecology and evolution. He received his PhD. in Botany from the University of Michigan in 1987, and after a brief postdoctoral position at the University of California Berkeley, he joined the faculty there in 1989. His best known work is focused on mycorrhizal fungi. These fungi form symbiotic associations with plant roots, and this interaction represents one of the most widespread and important mutualisms in terrestrial ecosystems. Early work on mycorrhizal fungi from the Bruns lab includes: i) the development of molecular tools for the identification of fungi from environmental samples; ii) the characterization of fungal community structure; iii) the effect of plant host and disturbance on fungal community structure; iv) the autecology and population structure of key fungal species; and v) the ecology and evolution of non-photosynthetic, epiparasitic plants and their fungal hosts. All of this work has been facilitated by using molecular methods, such as nucleotide sequencing of environmental samples, as a means of identifying the participants involved in these interactions.

Current work in the Bruns lab includes: 1) the roles of dispersal limitation and competition in structuring ectomycorrhizal succession; 2) composition and global patterns of indoor fungal assemblages; 3) composition and structure of fungal assemblages associated with the biodegradation of bioenergy crops, and 4) genomics of *Rhizopogon salebrosus* species in the context of epiparasitism by *Pterospora andromedea*. In addition Bruns is a founding member of the Fungal Environmental Sampling and Informatics Network (FESIN). Its primary goals are to coordinate and facilitate information retrieval for fungi derived from environmental settings, and to help expand the field of fungal ecology through educational innovation.

THE PROMISE AND CHALLENGE OF ENVIRONMENTAL SEQUENCES - AN EXAMPLE FROM THE FUNGI

Thomas Bruns
University of California, Berkeley, California, USA

Given the current trends in high-throughput sequence acquisition the bulk of information about taxon distributions and sequence variability for most small organisms (e.g., bacteria, fungi, microinvertebrates) will be connected to environmental sequences within the next decade. To realize the full potential of these data we need to develop rapid ways to validate and classify them.

Fungi provide an excellent example of the impact of environmental sequences as roughly a third of all fungal ITS sequences in GenBank are derived directly from substrates such as soil or plants. These sequences are not connected to specimens and are often crudely identified because no close matches to sequences derived from specimens or cultures are available. Barcoding alone is unlikely to improve classification of these sequences because many of the taxa are not frequently collected or cultured even though they may be abundant in the ecosystems from which they are derived. For these reasons a naming system is needed for environmental sequences in order to connect the information about the underlying taxa. Naming of environmental sequences is potentially more straightforward than with specimen-based barcoding, because it is not coupled directly to species names. Instead names could be numeric and clade-based, and the potential exists to make the process at least semi-automated by making use of phylocode-like conventions to delimit terminal clades.

Validation of environmental sequences is a second problem, as these sequences are subject to unique errors, such as chimeras, and it is more difficult to ascertain their quality or to resequence them for verification. However, as data accumulate comparison across studies is becoming a powerful way to determine valid sequences. By limiting naming of environmental sequences to those that recur across studies one could focus on a validated set of sequences that are linked to the most data about distributions and habitats.

Plenary Session Speakers & Abstracts

SHADI SHOKRALLA

Shadi Shokralla is originally from Egypt. He obtained his MSc and PhD from Microbiology Department, Faculty of Pharmacy at Mansoura University (Egypt) and in collaboration with Stanford Genome technology Center at Stanford University, USA. During his work at Stanford, he used different molecular techniques to detect microbial resistance amongst clinical isolates of bacteria. He focused his research on the DNA sequencing methods and tried to optimize sequencing-based approaches for standard identification of biological specimens. He is interested in technology development projects especially the application of next generation sequencing technologies in addressing challenging questions that are not feasible using Sanger sequencing approach. In 2008, he joined Biodiversity Institute of Ontario as a postdoctoral fellow to work on environmental barcoding approach using next-generation sequencing. Besides keeping their 454FLX machine running smoothly, he is currently working on developing new molecular approaches for total biodiversity assessment from different environmental samples. He has been developing robust primer sets and amplification strategies for uniform sequencing of different groups of organisms using 454. He is very interested to further hone his skills and learn about data analysis and bioinformaics.

PYROSEQUENCING FOR RAPID MINI-BARCODING

Shadi Shokralla (1), I. Meusnier (1), D. Janzen, W. Hallwachs, P. Hebert (1) &M. Hajibabaei (1)

(1) Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada
(2) University of Pennsylvania, Philadelphia, Pennsylvania, United States

DNA barcoding is an effective approach for species identification and for discovery of new and cryptic species. Sanger sequencing technology is the method of choice for obtaining standard 650bp cytochrome c oxidase I (CO1) barcodes. However, DNA degradation/fragmentation makes it difficult to obtain a full-length barcode from old specimens. Mini-barcodes of 130 bp from the standard barcode region have been shown to be effective for accurate identification in many groups and may be readily obtained from old samples of up to 200 years old. Here we demonstrate the application of single-specimen four enzyme pyrosequencing technology (PSQ) in rapid, cost-effective mini-barcode analysis. Pyrosequencing relies on sequencing by synthesis through detection of inorganic pyrophosphate release on successful nucleotide incorporation. We were able to generate sequences of up to 100 bp from within the barcode region of CO1 in a specimen of each of 50 species of Lepidoptera. The sequences obtained using pyrosequencing were of high quality and we were able to robustly match all the tested samples to their respective Sanger-sequenced standard barcode sequences. Simplicity of the protocol and instrumentation coupled with higher speed and lower cost per sequence than Sanger sequencing makes this approach potentially useful in efforts to link standard barcode sequences from unknown specimens to known museum specimens blessed with only short DNA fragments.

Plenary Session Speakers & Abstracts

MICHAEL RHODES

Michael Rhodes graduated from York University with a degree in Genetics, did a Ph.D. in bioinorganic chemistry at University of London. After a post doc in Chicago working on genetics of metal transport in P. aeruginosa, returned to UK to work at United Kingdom Human Genome Mapping Project Resource Centre, finishing as Operation Manager in charge of four teams: Mouse resequencing, Linkage Hotel, Academic Services and Custom services. Joined Applied Biosystems in 1999, worked on Genotyping (Linkage mapping set, SNaPshot, Taqman assays, SNPlex and GeneMapper). Spent the last few years working on SOLiD because "the sequence is the ultimate genotype"

BARCODING PRESENT AND FUTURE

Michael Rhodes

Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94401

With the tremendous progress of the Barcode of Life project, two challenges seem apparent to an outsider. How to distinguish and identify species when the CO1 (or any barcode) region does not show enough discrimination? How can the barcode reference library be utilized in practical applications beyond academic labs? Up until now the bulk of discovery has been carried out using capillary electrophoresis. The advent of next generation sequencing technologies has led to the possibility of vastly reduced sequencing costs in the genomic laboratory. One important question is how these capabilities can be used by the Barcode for Life project. Is it possible and cost-effective to use a 100Gigabase sequencer in such a project? Already SOLiD system has been used to differentiate between closely related bacterial strains and develop assays for these strains. As well as whole genome sequencing the rapid advances in enrichment technologies (Agilent, Febit, Raindance, Nimblegen, O-link) in conjunction with highly multiplexed runs have raised the possibility of running multiple samples and benefiting from the high throughput of the SOLiD System. Data will be presented on targeted resequencing and also on bacterial discrimination.

The ability to discriminate based upon sequence differences has been used in multiple quality and safety kits, some of the newer kits will be discussed and how barcode results could be implemented into new assays.

The new open array system will be presented: a medium-format SNP genotyping system (15-256 SNPs) aimed at those labs scoring populations for the presence of particular variants.

Finally the present status of capillary electrophoresis (CE) will be reviewed because CE is still the workhorse when there are few samples with short sequence regions of interest. The newest platform is now a bench-top device designed for ease of use.

NEOTROPICAL BIRDS BARCODING: A JOINT INITIATIVE

Barreira, A.S.(1), Gómez, M.I.(2), BENITES, P.(1), Kopuchian, C. (1), Naoki, K. (3)

(1) Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina
(2) Colección Boliviana de Fauna, La Paz, Bolivia
(3) Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, Bolivia.

The project "Barcoding the birds of Argentina" started in 2005 and the DNA Barcodes for 573 species (2087 specimens) were obtained so far. Some species, many of them distributed also beyond the Argentine border, showed some genetic divergence but a larger intraspecific structuration is expected if the study is extended to a regional scale. Therefore, as part of a second phase of the project, a joint effort between the national ornithological collections of Argentina and Bolivia was initiated in 2008 with the objective of building a tissue collection of the birds of Bolivia, with their corresponding identification vouchers, and obtaining their DNA Barcodes. Around 1400 bird species inhabit Bolivia and only 50 % of them are shared with Argentina; therefore the study of its avifauna results essential to increase the amount of species present in the ABBI database and to study the intraspecific genetic diversity in a larger scale.

Two collecting expeditions were conducted in areas of Yungas and Amazonia in La Paz department, and we collected 311 specimens of 127 different species, 53 of which are new for the ABBI dataset. So far 109 DNA Barcodes from 63 different species were obtained. When these sequences were compared to other sequences of the same species deposited in BOLD, we found some cases of large genetic divergence. The most striking one was the case of the Red-crowned Ant-Tanager (*Habia rubica*), with the populations from Argentina and Bolivia having a mean genetic distance of 7.2 %, which is even larger than the mean nearest congener distance found for the first 500 species analyzed for the birds of Argentina (6.2 %).

These preliminary results indicate that the avifauna from Bolivia can add many new species to the ABBI dataset and also reveal cases of deep intraspecific genetic divergence suggesting the presence of cryptic species among Neotropical birds.

COI FLAGS A RECENT RADIATION IN PASSERINE BIRDS

CAMPAGNA, L. (1), Lijtmaer, D.A. (1), Lougheed, S.C. (2), Tubaro, P.L. (1)

(1)Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Ciudad de Buenos Aires, Buenos Aires, Argentina
(2)Queen's University, Kingston, Ontario, Canada

The capuchinos comprise a group of 11 *Sporophila* species with little morphological differentiation, extended sympatry, and marked sexual dimorphism in both color patterns and vocalizations. Our previous work using mitochondrial DNA (partial cytochrome *b* and COII-Tlys-ATP8 fragments)

suggested that the capuchinos are monophyletic and further divided into two clades: northern capuchinos (2 species) and southern capuchinos (9 species). The phylogenetic relationships among the southern capuchinos were unresolved due to lack of reciprocal monophyly among species. The objective of the present study was to clarify the phylogenetic relationships of the capuchinos using an augmenting data set of DNA sequences. In total we sequenced 4.2 kb (cytochrome *b*, cytochrome *c* oxidase 1 (COI), mitochondrial control region and 2 nuclear pseudogenes: *numt2* and *numt3*) and genotyped 191 samples for 6 DNA microsatellite loci. We included COI to permit us to evaluate its efficacy in diagnosing species within this challenging group. We subjected the sequences to neighbour-joining, maximum parsimony and Bayesian phylogenetic analyses and estimated levels of differentiation among species using frequency-based analyses of microsatellites. We found that the southern capuchinos share COI haplotypes and show extremely low interspecific divergence constituting the only example of several avian species that cannot be separated among the neotropical birds barcoded thus far. No further insight into the phylogenetic relationships of the group was obtained with the remaining markers, suggesting that the lack of resolution using COI is due to the unique evolutionary history of the southern capuchinos rather than to limitations of this molecular marker. Our results flag the southern capuchinos as a new case of an explosive and recent radiation where incomplete lineage sorting and hybridization with introgression are the most likely causes for the observed genetic pattern.

BARCODING AGAMID LIZARDS IN VIETNAM

CHE, J. (1), Jin, J.Q. (1), Grazziotin, F.B.(2), Nguyen, S.N. (1), Zaher, H. (2), Orlov, N. (3), Ananjeva, N.B. (3), Murphy, R.W. (1,4), and Zhang, Y.P. (1)

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Agamid lizards form a dominant component of the herpetofauna throughout their range. Being diurnal, many species are commonly encountered by local residents. Some species are used as food and maintained as pets, yet others are rarely encountered owing to arboreality and crypsis. We undertook a barcoding analysis of species and populations in Vietnam while using global representative taxa. We surveyed about 120 species representing seven genera. Intergeneric divergence was sufficient to unambiguously assign unknown samples to genera. Within genera containing multiple species, interspecific divergence allowed the correct identification of species. Intraspecific divergence was significant in some species indicating the possibility of multiple cryptic species.

UNRAVELING THE FOOD WEB OF AN INSECTIVOROUS BAT COMMUNITY

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One of the most difficult interactions to observe in nature is the relationship between a generalist predator and its prey. For insectivorous bats, direct observations are often impossible so we rely on the morphological identification of digested prey remains making species-level identification extremely difficult. These investigations rarely provide identifications beyond order or family, severely restricting our understanding of ecological relationships.

We previously used residual mtDNA in fecal pellets as a means to generate DNA barcodes used to identify prey of the bat *Lasiurus borealis* (Clare et al. 2009). Here we extend this to include insects eaten by eight sympatric bat species in Ontario, Canada (*Myotis lucifugus, M. leibii, M. septentrionalis, L. borealis, L. cinereus, Lasionycteris noctivagans, Eptesicus fuscus* and *Perimyotis subflavus*). The resulting community food web includes more than 300 species of insect, spider and bat confirmed by DNA barcoding. With many hundreds of distinct connections this represents one of the largest molecular food webs ever constructed.

Using this food web, we address issues of resource partitioning in this community of bat species and variations in the predator-prey relationships. With the specific example of five months of intensive dietary monitoring of *L. borealis* and *M. lucifugus,* we have identified seasonal patterns in the food web coinciding with physiological demands associated with the bats' periods of parturition, mating and preparation for hibernation. Analyses of spatially separated collecting sites also demonstrate shifts in diet associated with colonies in urban versus natural habitats.

The application of DNA barcoding beyond taxonomic investigations is a new and exciting field of research. This investigation demonstrates the power of DNA barcoding libraries for the practical application of unraveling complex ecological community dynamics.

HIGHLIGHTS OF THE BIRDS OF MEXICO PROJECT

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Mexico has the largest number of endemic bird species per land area, and within its limits it comprises both temperate and tropical habitats, hence barcoding this fauna is interesting within the ABBI campaign. So far, resident birds of Mexico have been sequenced for CO1 with 661 sequences and 255 species so far, using samples from the bird tissue collection at the Institute of Biology at UNAM. Using the 2% parameter of neighbor joining distance in groups where we have several samples assayed, 11 species showed clusters

that deserve a more detailed examination. Many times these higher molecular distances within species correspond to subtle plumage differences in geographically separated groups that might actually have subspecies names. Together with other molecular and morphological markers we are exploring biogeographical differentiation in three lowland groups, and will present data for multilocus and non-distance analysis in them. These groups correspond to *Arremonops* spp, *Cyanocompsa parellina* and *Uropsila leucogastra* populations. Each lowland group is giving us a different biogeographic pattern perhaps due to dissimilarities in habitat or age differentiations.

DNA BARCODE RESOLUTION IN EASTERN PALEARCTIC BIRDS

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Birds, because of their mature taxonomy, remain an ideal group to test the efficacy of DNA barcode-based species assignment. COI diversity has been surveyed extensively in North American birds, but treatments elsewhere are so far sparse. In this study, we expand the avian COI library by sampling a large portion of the Palearctic avifauna. We acquired COI sequences from vouchered museum specimens (N=1674) representing 398 species and merged this data with that of North American birds for further analyses. Avian diversity is generally low in the Palearctic but a number of species are sister to Nearctic taxa and a few additional species are common to both regions. This allows transcontinental comparisons of both inter- and intraspecific divergence. We tested three different commonly used methods for species assignment including neighbour-joining clusters, distance-based thresholds (via the MOTU assignment program), and character-based methods (via the CAOS system). We compare the results and reliability of these tests and discuss the appropriateness of each in different cases. In general, most species could be easily delimited using at least one or a combination of the described methods. Roughly 96% of species could be accurately identified to species and average levels of divergence mirrored that observed in the Nearctic, despite it being a much smaller landmass with a different glacial history. Well-supported divergences within 44 species and their implications regarding species boundaries are discussed, as are reasons for limited diversity observed within 22 groups of species, mostly limited to pairs (e.g. recent speciation events, introgression, etc.).

BARCODING ANURANS FROM BRAZILIAN ATLANTIC FOREST

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Amphibian populations around the world are in decline and, at the same time, it seems that the number of amphibian species is highly underestimated, calling for an acceleration of taxonomic research. Identifying species of organisms by short sequences of DNA (DNA barcoding) can greatly facilitate this process and may provide a way to rapidly evaluate species richness. In amphibians, the utility of COI DNA barcoding has been discussed due to two main problems: 1- the high variability of priming sites that hinder the application of universal primers and 2-the difficulty in the definition of threshold values to identify candidate species. Alternatively, the use of 16S rRNA as DNA barcoding of amphibians is being suggested. Here, we conducted a preliminary analysis of COI as DNA barcoding for Anuran from Brazilian Atlantic forest. We have analyzed new COI and16S rRNA sequences from 58 different species of anurans, and also three caecilians and one caudata (~3 individuals per species). We proposed a new primers set for amphibians and were able to amplify COI and 16S fragments from all samples tested. The inter-specific divergences found were higher than 18% for both markers and there was no overlapping between intra- and inter-specific variations. High intra-specific COI and 16S divergences (> 7% and >5%, respectively) were observed in 12 of the 58 anurans analyzed. In most of these cases, geographically distant samples belong to genetically highly distinct lineages that could be considered as distinct species. These results suggest that the anuran diversity in Brazil may be highly underestimated. But these "new" species need to be validated by other independent lines of evidence and the threshold values to identify candidate species still need to be defined. Based on our data we concluded that both COI and 16S rRNA have a great potential for DNA barcoding amphibians. But we strongly suggest that a large-scale effort to barcode the amphibians, using the same primary barcode region of COI, may yield important findings for the knowledge and conservation of these species. The use of DNA barcode will help to make a more exact estimate of amphibian species richness and to formulate a more precise evaluation of global amphibian decline, which may be worse than previously realized.

NEW TOOLS TO STUDY SPECIATION AND COMMUNITY ECOLOGY: EXAMPLES FROM PANAMA'S BIRD BARCODES

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Horizontal surveys across species assemblages, employing a standard genetic marker and using standard sampling procedures provide powerful tools for basic studies in ecology and evolutionary biology. DNA barcoding is, to date, the largest such survey, and researchers are only beginning to realize the full import of such massive datasets. Here we present findings from our soon-to-be completed survey of mtDNA barcodes of the birds of Panama, a country with the eighth richest

avifauna in the Americas. We focus on how such data can inform our understanding of the speciation process and community ecology. Many species of Panamanian birds are comprised of multiple mtDNA clusters with k2p genetic distances as high as 1 – 6%. These clusters are monophyletic and appear to be the result of phylogeography, i.e. historical isolation of populations, rather than introgression or hybridization with other species. While some of these cases may represent cryptic species diversity, the broad overlap of divergent clusters in other cases suggests that specific cohesion is maintained despite high levels of mtDNA divergence. Analyzing these patterns from a community perspective provides other insights. In most cases, a species from an avian community in Panama differs by at least 6% and more often between 8 – 12% k2p distance from the most closely related next species in that community. This minimum distance to closest community member appears to be slightly higher than for birds from North America, at least in passerines where it is easiest to make direct comparisons. Finally, when we analyze our data in spatially-nested sets, we found little evidence for the competitive exclusion of closely related taxa from local species pools, even among avian communities with high species richness.

DNA BARCODES AGAINST THE ILLEGAL PARROT TRADE

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In 2003 an international smuggler was caught in a Brazilian airport with 58 avian eggs. He alleged that they were quails, but the police authorities suspected that they were parrot eggs. As 25% of parrot species are endangered, the identification of the eggs' corresponding species was important to establish a stronger case against the smuggler. The embryos never hatched; thus, it was not possible to identify by morphology. The aim of the present study was to identify the embryos' species based on DNA barcoding. The sequences obtained were compared to all avian sequences deposited in BOLD (The Barcode of Life Data System) using the Identification System search. Additionally, all parrot sequences from BOLD were used to obtain neighbor-joining trees based on Kimura 2 parameters' model of molecular evolution. Three samples were identified as *Ara ararauna*, two as *Triclaria malachitacea*, and 49 as *Alipiopsitta xanthops*. The four remaining samples were identified as belonging to the complex *Amazona aestiva/Amazona ochrochephala*. All these species are Neotropical parrots that occur in Brazil. As the investigation indicated that the smuggler had only been in Brazil, our results are congruent with this scenario. This is an example where DNA barcoding was successfully applied to identify species of birds apprehended from the illegal animal trade and showed to be a powerful tool to help the conservation of target species. Funds (Brazil): FAPESP, CNPq, and CAPES.

DISTANCE-BASED AND CHARACTER-BASED APPROACHES TO BARCODING TURTLES

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Molecular barcoding has the potential to serve as a powerful tool in wildlife forensics and may prove to be a vital aid in conserving organisms that are threatened by illegal wildlife trade, such as turtles (Order Testudines). Novel COI sequences (650 bp) were produced for a set of 183 turtle species and combined with publicly available sequences for an additional 36 species to produce a data set representative of the breadth of the order. Variability within the barcode region was assessed and the utility of both distance-based and character-based methods for species discrimination was evaluated. For species in which genetic material from more than one individual was available (n=66), intraspecific Kimura 2-parameter divergences were 1.3% on average, although divergences greater than 2% occurred within 14 species. High intraspecific divergences could indicate species with a high degree of internal genetic structure or possibly even cryptic species, although introgression is also likely in some of these species. Divergences between species of the same genus were 6.4% on average; however, 48 species were less than 2% divergent from congeners. Low levels of interspecific divergence could be caused by recent evolutionary radiations coupled with the low rates of mtDNA evolution previously observed in turtles. A character-based method for identifying diagnostic sets of nucleotides (the characteristic attribute organization system, or CAOS) performed better than distance-based methods for distinguishing taxa in genera with low levels of interspecific variability. Seventeen species (7.8% of all species evaluated) could not be distinguished using either diagnostic characters or the customary 2% distance threshold. In general, the sequence information obtained in this investigation will likely be a valuable aid in conserving the turtle species studied here, most of which are highly threatened.

DIGITAL INDICATOR VECTORS MAP GENETIC DIVERSITY IN SOUTH AND NORTH AMERICAN BIRDS

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Large, newly-available DNA barcode data sets offer the possibility of analyzing a uniform locus across a broad diversity of life forms. We calculated digital indicator vectors characteristic of taxonomic groups, using a 501 bp segment of cytochrome c oxidase subunit I (COI) gene, and applied this approach at two levels of organization, 12 widely-separated animal groups and 630 South and North American bird species. The approach was computationally efficient and enabled accurate assignment of over 10,000 test sequences. The structure matrix of vector correlations displayed in a 2D graphic quantified diversity among and within sets. Fractures in structure

matrices corresponded to boundaries among taxonomic divisions, and raised questions about existing classifications. The indicator vector analysis enabled visualization of discontinuous genetic structure of biodiversity at a wide range of scales, with the potential of enabling automated classification.

REALISING THE SCOPE FOR BARCODES IN BIOSECURITY

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In 2003 COI DNA barcode identification of quarantine insect pests was being considered in New Zealand. By 2005 it was employed on a routine basis for diagnosis of the economically highest risk groups, tephritid flies and lymantriid moths. Now, as DNA barcode libraries grow, a role for barcoding in a wider biosecurity context is becoming evident.

On the one hand diagnosis of a broader range of pest species is becoming possible, particularly for lepidopteran incursions due to the mounting volume of data in BOLD. On the other hand, inability of the method to discriminate some taxonomically cryptic species (possibly due to the selection of the incorrect model of molecular evolution) has fueled independent concerns about the taxonomic validity of some species; this not only has serious implications for trade, but has also stalled the development species-specific control methods such as SIT and surveillance tools based on pheromone lures. In contrast, the exposure of sub-specific divergences within gypsy moth, yellow peach moth and fall web worm, that correlate with other lines of evidence inferring morphologically cryptic species, has had positive outcomes for quarantine.

The senario's reviewed here illustrate how barcoding contributes to a broad range of biosecurity activity. Barcoding has value as a generic tool in a plant health diagnostic laboratory and offers a level of international standardisation and transparency that is difficult to achieve with other methods.

DNA BARCODES FOR THE PROFILING OF MICROBIOTA IN ENVIRONMENTAL SAMPLES

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To maintain sustainable farming systems, there is a necessity to improve knowledge, understanding and awareness of biodiversity of agriculturally important microorganisms. Discriminating and identifying species of fungi and bacteria from environmental samples has always been challenging, because traditional approaches are time consuming, labor intensive and require specialized experts. However, with the unprecedented increase of DNA sequences in public and in-house databases, and the development of innovative molecular technologies, DNA barcode-based approaches significantly enhance our ability to study agricultural microbial

community structure and to monitor crop pathogens. Microcodes derived from full length DNA barcodes can be used to design oligonucleotide arrays to provide massive scans of expected microbes in environmental samples. In the Biodiversity group of AAFC in Ottawa, DNA arrays have been designed or are under development for species of Cranberry fruit rot fungi, Pythium, Fusarium, Penicillium and Phytophthora. The steps required to design species/clade-specific microcodes have been automated with in-house and commercial computer programs. Arrays containing microcodes from multiple gene regions have improved detection accuracy and redundancy. Some arrays have been used for routine field applications by AAFC and Canadian Food Inspection Agency (CFIA).

We are now applying next-generation 454 pyrosequencing technology to amplicon libraries of barcode regions of air/rain-borne fungi and bacteria collected from a Canada-wide spore trapping network. We will generate comprehensive DNA barcode data on Canada's microbial biota and significantly expand the range of documented organisms. The aim is to contribute to a healthy agroecosystem by improving our capacity to understand the potential impact of climate change on microbial distribution, and the ability to immediately detect agriculturally important alien and invasive species in the field and on imported and exported commodities.

HOST TRACKING, CRYPTIC ADAPTATION? A BARCODE STUDY OF A PARASITOID OF THE HORSE CHESTNUT LEAFMINER

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Recent DNA barcoding of generalist parasitoids has revealed complexes of cryptic species. Here we study the eulophid Pediobius saulius, a pupal parasitoid of the highly invasive horse-chestnut leaf-mining moth, Cameraria ohridella. This parasitoid has a broad range of hosts, however no study has addressed the possibility of cryptic species within this alleged polyphagous parasitoid species. We DNA-barcoded 180 individuals from different hosts and geographic origins. DNA barcodes strongly support the existence of at least two highly differentiated parasitoid complexes, one Balkanic highly specialized that mainly (but not only) attacks C. ohridella, and a more generalist European one which include many hosts, including some C. ohridella. Barcode divergence (up to 4%) shows the existence of cryptic species, and this is confirmed by nuclear divergence (28S). The presence of a cryptic species localized in the Balkans attacking mainly Cameraria ohridella opens the possibility of further studies to assess its potential as biocontrol agent of this highly invasive moth.

SELECTING PRIORITY TREE SPECIES FOR DNA BARCODING IN AFRICA

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There are no less than 10,000 tree species in Africa, though the biodiversity of the continent remains to be fully documented. Identifying priority species from this myriad of taxa according to the general utilization, conservation and ecosystem services is paramount to the pursuance of the MDGs and sustainable development.

The application of biodiversity informatics to access the trees revealed 1,045 species of concern distributed across Africa, comprising 21 invasive alien species (IAS), 100 industrial wood species, 880 globally threatened species (870 uncategorized;10 CITES-listed) and 44 fruit species.

Marginal populations of several land races, cultivars and endangered species are held in home gardens with only limited research on their genetics and systematics needed to characterize their diversity. The characteristics of releases and spread of invaders follow closely the patterns advanced by the simple-demographic, spatial-phenomenological and spatial-mechanistic models. Invasiness is difficult to identify by the conventional methods during the lag phase but easy to control while during the explosion phase it is readily recognizable but difficult to eradicate. Biological invasions are widespread, constituting one of the main factors in biodiversity loss and endangered species listing in Africa. Trade, travels, ecotourism and globalization are implicated in plant species invasion. An efficient monitoring mechanism of illegal trade in CITES-listed specimens and other categories of threatened trees is absent in most ports and borders.

A systematic assessment of the genetic variability of the fruit trees is important for diagnosis and the selection of appropriate variants for domestication.
Accurate identification of the trees of Africa requires DNA-based species diagnosis, barcode, to enhance current efforts on the control and eradication of IAS, monitoring of trade in endangered species, utilization and conservation of genetic diversity of the fruit trees and management of the ecosystems for sustainable development in the continent.

Barcoding Species For Quarantine/ Plant Protection

MIND THE GAP: BARCODES AND DIAGNOSTIC STANDARDS

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In agriculture the threat of invasions leading to reduced profitability and loss of market access are key issues for those responsible for preventing entry and responding to border detections and incursions. Responsibility for detection of pests before they arrive, at the border or once they have established, is spread from the regulator through to the public. However, identification is largely the role of the regulator where the need for timely and accurate identifications is a key precursor to determining whether inbound goods are treated, rejected or allowed entry, and the level of response to a detection within a jurisdiction's borders. DNA barcoding provides diagnostic solutions for many of the threat taxa. However, there is a significant gap between current data standards and those required by national regulators under the International Plant Protection Convention. We discuss the requirements of a robust diagnostic system and compare these with (a) the requirements for accreditation of diagnostic protocols and diagnostic laboratories in Australia under SPHDS (Subcommittee on Plant Health Diagnostic Standards) and NATA (National Association of Testing Authorities) and (b) current draft DNA barcode standards. In Australia, SPHDS works through a series of working groups:

• Diagnostic Standards Working Group, whose role is identification, facilitation, assessment, approval and review of national diagnostic standards for emergency plant pests.

• Expertise and Resources Working Group, whose role is designation of quality assured national reference laboratories and collections.

• Accreditation Working Group works with NATA to develop, manage and audit the plant laboratory accreditation scheme and developing proficiency testing arrangements that underpin the laboratory accreditation scheme.

We discuss possible ways forward to ensure greater acceptance of DNA barcodes as a diagnostic standard used in the regulation of national biosecurity.

SOIL NEMATODE DIVERSITY AND COMMUNITY COMPOSITION AS A BASIS FOR BIOSAFETY ASSESSMENT OF TRANSGENIC BANANA IN THE FIELD

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Although, information on relatedness in nematodes is commonly obtained by DNA sequencing of the ribosomal internal transcribed spacer (ITS) region, the level of diversity in the ITS loci has often proven insufficient for reliable species differentiation. Recently, the sequences of a fragment of the small subunit nuclear ribosomal DNA (SSU or 18S-rRNA) was shown to be more useful in identification of nematodes and other small organisms. We are currently developing a database of nematode species in Uganda using the sequences from SSU. We will use this information as a molecular marker for environmental monitoring of the effects of transgenic bananas bearing transgenes for resistance to parasitic nematodes of banana and other biotic constraints. To-date, we have sequenced individual nematodes sampled from a banana plantation in a plot designated for a confined field trail (CFT) of transgenic bananas at National Agricultural Research Laboratories in Uganda. From this initial screening, search for relatedness in the public genomic database identified 22 different nematode species. Common nematode species identified included *Radopholus similis Helicotylenchus multicinctus* and *Helicotylenchus dihyslera*, *Aphelenchus avenae*, *Rotylenchulus reniformis*, *Rotylenchus uniformis*, *Globodera* spp., *Acrobeloides* spp., and *Acrobele thornei*. Further establishment of the identities will be done by characterizing the same DNA samples using the universal primers developed from the mitochondrial Cytochrome C Oxidase subunit I (COI) gene, recommended for DNA barcoding by the Consortium for the Barcode of Life (CBOL, www.barcoding.si.edu). To initiate the biosafety analysis, we are currently sequencing DNA from nematodes collected from the same plot currently containing a CFT of transgenic banana developed for resistance to Black sigatoka disease (*Mycosphaerella fijiensis*) by over expressing the antifungal chitinase gene from rice. Differences in nematode diversity and community composition will be used to assess the impact of transgenic banana on non target organisims and thus on the overall environment.

KEY WORDS; Nematodes, Biosafety assessment, environmental biomarkers, barcoding

COLEOPHORA (LEPIDOPTERA, COLEOPHORIDAE): ENHANCED SPECIES DISCOVERY AND TAXONOMY THROUGH DNA BARCODING

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We sequenced 3000+ specimens of Coleophora representing ~335 species, including 135 (75%) described and 180 undescribed from North America. DNA barcodes were highly effective in species recognition, resulting in the separation of nearly all species sampled. Barcodes correlated closely with existing species based on genitalia, host plants and larval cases. Shorter barcode fragments ('mini-barcodes') were successfully recovered from type specimens up to 136 years old and matched remarkably well with barcode sequences obtained from recent specimens. In a mega-diverse but morphologically uniform genus like Coleophora, DNA barcodes provide a highly effective means of enhancing the taxonomic workflow by successfully sorting large series of specimens, associating sexes and otherwise unidentifiable life stages, uncovering cryptic species, and reducing the need for time-consuming micro-dissections. Our framework incorporates DNA barcoding with traditional character systems for an integrative approach applicable to Microlepidoptera taxonomy, which could significantly reduce the current impediment caused by an inadequate taxonomic foundation and the need for large-scale micro-dissections.

THE DNA BARCODE LINKER

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DNA barcoding is based on the use of short DNA sequences to provide "taxonomic tags" for rapid, efficient species identification. Currently, reference databases are being compiled. In the future, it will be important to facilitate access to these databases, especially for non-specialists.

The method described here provides a rapid, web-based, user-friendly link between the DNA sequence from an unidentified biological specimen and various types of biological information, including the species name. Specifically, we use a customized, Google-type search algorithm to quickly match an unknown DNA sequence to a list of verified DNA barcodes in the reference database. In addition to retrieving the species name, our program also provides automatic links to a range of other information about that species.

As the DNA barcode database becomes more populated, it will become increasingly important for non-specialists to be able to exploit it for the rapid identification of unknown specimens and to easily obtain relevant biological information about these species. The method presented here meets that need.

THE BLOG SYSTEM: LOGIC DATA MINING FOR COMPACT EXPLANATORY SPECIES CLASSIFICATION

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A logic classification method based on logic and integer programming has been applied to the problem of species classification through barcode. The adopted Logic Mining method is based on the solution of a extended set covering problem and a sequence of minimum cost satisfiability problems. The method has exhibited high correct recognition rate on training-testing splits and is able to identify a small number of the loci represented in the barcode strings as support set of the logic classification formulas. The system has now been integrated in a software tool, the BLOG system (Barcoding with LOGic), to be made available for on-line classification of barcode fragments in the CBOL website. In this presentation we describe the mathematical foundations of the method and several new techniques specifically developed to interpret the results of the barcode analysis. We finally discuss the results obtained with the proposed method on barcode data from different species and compare our results with those obtained by alternative barcode analysis methods.

LINKING DNA BARCODES AND BIODIVERSITY INFORMATION: THE BIODIVERSITY INFORMATION SYSTEM OF MEXICO

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The information about biodiversity is massive, fuzzy and highly heterogeneous in concept, scale and time. Furthermore, almost any biodiversity centered system has to tainted their concepts to a broader representation to include environmental and social information, in order to achieve some real value for scientific synthesis, rapid response and early warnings to sustain increasing decision makers demanding such information.

Accordingly, the National Biodiversity Information System (SNIB) has expanded its boundaries to their inception to a biotic, abiotic, environmental and social consist of component system, within a species backbone, primary based on biological collections and observational data. Biodiversity "untainted" components in the SNIB are those classical description which include genes, species and ecosystems. The primary data naturally has to key components the taxo-reference and the geo-reference, but also to additional information. Even these apparently structured data gone to fuzzy, access and linking mechanism into an information system depends on the questions and the perspective, which demands clear ways to integrate the information. SNIB's interoperability rank from human knowledge, made case by case, to automatic computer provided.

The SNIB is not a finished system, tested every day based on production of several decision relevant products from checklists, species information, highly integrative analysis to rapid response systems. These products show clearly how integration should tight and shape a Biodiversity+ system. CONABIO as part of the Mexican commitment to construct the DNA barcode processes, is supporting several DNA barcode researcher projects and has drawn a initial integration process to the SNIB and envision some ideas on the de facto utilization to support decision, like biodiversity new measures, link with the species name in the SNIB and other systems like EOL, REMIB, GBIF and combining analysis with others systems like BOLD.

ERROR RATES OF PHYLOGENETIC AND SUPERVISED CLASSIFICATION ALGORITHMS IN DNA BARCODING

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DNA barcoding aims to assign individuals to given species according to their sequence at a small locus, generally part of the CO1 mitochondrial gene. Amongst other issues, this raises the question of how to deal with within-species genetic variability and potential transpecific polymorphism. In this context, we examine several assignation methods belonging to two main categories: (i) phylogenetic methods (neighbour-joining and PhyML) that attempt to account for the genealogical

framework of DNA evolution and (ii) supervised classification methods (k-nearest neighbour, CART, random forest and kernelmethods). These methods range from basic to elaborate. We investigated the ability of each method to correctly classify query sequences drawn from samples of related species using both simulated and real data. Simulated data sets were generated using coalescent simulations in which we varied the genealogical history, mutation parameter, sample size and number of species.

Results: No method was found to be the best in all cases. The simplest method of all, "one nearest neighbour", was found to be the most reliable with respect to changes in the parameters of the data sets. The parameter most influencing the performance of the various methods was molecular diversity of the data. Addition of genetically independent loci - nuclear genes - improved the predictive performance of most methods.

Conclusions: The study implies that taxonomists can influence the quality of their analyses either by choosing a method best-adapted to the configuration of their sample, or, given a certain method, increasing the sample size or altering the amount of molecular diversity. This can be achieved either by sequencing more mtDNA or by sequencing additional nuclear genes. In the latter case, they may also have to modify their data analysis method.

EFFICIENT ALIGNMENT-FREE BARCODE ANALYTICS

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Alignment-free DNA barcoding is a new approach to analysis of large volumes of barcode data, inspired by recent developments in the fields of pattern recognition and machine learning. In contrast to traditional phylogenetic methods which require computationally demanding analysis of complete and aligned barcode sequences, a typical alignment-free method treats barcodes as collections of short sequence fragments. This simpler representation is computationally efficient and scalable and offers a principal way to incorporate heterogeneous data sources (e.g., image-based morphology, GIS) into the barcode analysis. In this work we illustrate that alignment-free methods not only result in highly accurate and fast species identification models, but also offer alternative data visualization means. As a result, alignment-free barcode analytics provide a useful complement to traditional phylogenetic approaches.

The alignment-free methods compare fragment frequencies, the so-called sequence spectra, to quickly assess similarity between any two barcodes. These pairwise similarities enable one to rapidly analyze large sets of barcodes and classify queries, or to perform clustering analyses of putative species barcodes and display alternative visualizations for taxonomic groups, e.g.

projections of trees onto 2D planes.

We demonstrate the utility of alignment-free analytics on several benchmark barcode datasets, including ACG, Hesperiidae, Fish larvae, and Birds of North America, where they considerably improve species prediction accuracy and running time compared to prior results. We also illustrate applications of alignment-free methods to the new species detection, as well as for clustering analysis of putative barcode sample collections, and identification of critical within-barcode loci distinguishing barcodes of different sample groups.

ASSIGNING SEQUENCES TO SPECIES IN THE ABSENCE OF A BARCODING GAP

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Barcoding is an initiative to define a standard fragment of DNA to be used to assign unknown sequences to existing known species groups that have been pre-identified externally (for example, by a taxonomist). Several methods have been described that attempt to place this assignment into a Bayesian statistical framework. Here we describe an algorithm that makes use of segregating sites and we examine how well these methods perform in the absence of an interspecific barcoding gap. When a barcoding gap exists, that is when the data are clearly delimited, most methods perform well. Here we have used data from the *Drosophila* genus because this genus includes sibling species and the species relationships within this species complex are, arguably, better understood than in any other group. The results show that the Bayesian methods perform well even in the absence of a barcoding gap. The sequences from *Drosophila* are correctly identified and only when the degree of incomplete lineage sorting is extreme in simulations or within the *Drosophila* species do they fail in their identifications and even then, the ``correct'' species has a high posterior probability.

VISUALIZING AMINO ACID VARIATION IN THE CO1 BARCODE REGION

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The DNA barcode, although primarily focused on DNA, represents a wealth of information about the Amino Acid makeup of the barcode region of Cytochrome Oxidase 1. Studying the amino acid variation present in the barcode region opens the door to improved automated alignment of barcode data, better quality control, and an increased understanding of when sequence variation

has a functional impact on CO1. With these goals in mind, we present a tool for analyzing and visualizing amino acid variation in the barcode region.

Our tool has two primary functions: mapping AA variation and properties onto the CO1 secondary structure, and to aid in constructing a barcode specific AA substitution matrix. Mapping the AA variation onto the CO1 secondary structure allows us to identify hot spots of variation in the barcode region.

Constructing a barcode specific AA substitution matrix aids in both alignment and quality control of barcode sequences. By characterizing which AA substitutions are rare, and which are common in the barcode region we can easily identify aberrant substitutions for further investigation.

We demonstrate the functionality of our tool on published barcode data from both Hymenoptera and Lepidoptera and are currently working to integrate the tool directly into the Barcode of Life Data System.

DIGITAL INDICATOR VECTORS REVEAL DISCONTINUOUS GENETIC STRUCTURE OF BIODIVERSITY

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Large, newly-available DNA barcode data sets offer the possibility of analyzing a uniform locus across a broad diversity of life forms. We calculated digital indicator vectors characteristic of taxonomic groups, using a 501 bp segment of cytochrome c oxidase subunit I (COI) gene, and applied this approach at two levels of organization, 12 widely-separated animal groups and 630 South and North American bird species. The approach was computationally efficient and enabled accurate assignment of over 10,000 test sequences. The structure matrix of vector correlations displayed in a 2D graphic quantified diversity among and within sets. Fractures in structure matrices corresponded to boundaries among taxonomic divisions and raised questions about existing classifications. The indicator vector analysis enabled visualization of genetic structure of biodiversity at a wide range of scales, with the potential of enabling automated classification.

A TOOL FOR IDENTIFYING DIAGNOSTIC DNA CHARACTERS

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The technique of identifying a specimen to species using a standard gene region, DNA barcoding, is being successfully employed across the animal kingdom with recent advances in identifying a standard region for other kingdoms. DNA barcode data are employed in species identification using comparison-based, distance-based, and character-based approaches. Character-based species identification refers to the determination of single or compound characters (conventional

morphological, behavioural, genetic or other) that are fixed in one taxon and differ from others. We introduce the program, Diagnostica, which employs a novel character-based method for locating DNA sequence characters that are diagnostic between taxa. Here we describe DNA sequence characters as diagnostic if they consist of either single or compound characters at position(s) across aligned homologous DNA sequence data sets that can uniquely identify these data sets from one another. Diagnostica has been tested successfully on multiple DNA barcode data sets revealing its ability to locate diagnostic characters at multiple taxonomic levels.

ASSIGNING UNKNOWNS TO HIGHER TAXA USING DNA BARCODES

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When a specimen belongs to a species not yet represented in a DNA barcode reference library, there is considerable disagreement in the literature over the effectiveness of using sequence comparisons to accurately assign the query to a higher-taxon. Library species richness has been proposed as a critical factor affecting the accuracy of such assignments, but was never thoroughly investigated.

We explored the accuracy of assignments to genus, tribe and subfamily of 118 query species with five different assignment criteria, one distance-based and four tree-based. These Costa Rican species belong to Sphingidae; a family for which there is an almost complete DNA barcode reference library. An automated procedure was used to simulate different levels of species richness (10 to 100% of the available species) in reference libraries, and to record assignments (positive or ambiguous) and their accuracy (true or false based on current classification) under the five criteria.

Using a liberal tree-based criterion, an average of 80% of queries were accurately assigned a genus name with libraries containing 20% of available species, while 87% were accurately assigned a genus name with a library containing all available species. The liberal tree-based criterion assigned an average of 74% of queries accurately to tribes and an average of 90% accurately to subfamilies, across all libraries. The results suggest that the species richness of the reference library had only a weak effect on assignment accuracy, whereas which assignment criterion was used had a strong effect. Additional parameters in the tree-based criteria, such as exclusivity of taxa, decreased the number of false positive assignments, but also increased the number of false ambiguous assignments. Our findings suggest that barcode reference libraries can be useful for higher-taxon assignments long before the libraries achieve complete species richness.

BARCODING FRESHWATER FISHES: EXTENSIVE COVERAGE AND SUB-SPECIFIC IDENTIFICATION

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Traditional taxonomy is largely based on discontinuous patterns of morphological variation. As a consequence, testing the efficacy of DNA barcodes to identify known species relies on assessing the correspondence between clusters of mitochondrial haplotypes and morphological voucher specimens labelled as distinct species. Despite the plethora of published barcode studies to date, relatively few have achieved exhaustive species coverage for a given taxonomic group over a large geographic range. Specifically, the ability of barcodes to identify freshwater fishes (which are thought to hybridize frequently and often exhibit deep intra-specific divergence values) has not been fully assessed. Additionally, few studies have intentionally investigated the usefulness of barcodes to delineate distinct evolutionary lineages at the sub-specific level. In this context, we assembled the most exhaustive collection of barcodes for freshwater fishes to date. Represented by over 5800 specimens from Canada and the United-States, some 700 North American species (80% of the known species from this region) were analyzed. Extensive sampling of 9 co-distributed taxa, possessing different levels of morphological divergence and sub-specific designation, were further included to evaluate the performance of barcoding in distinguishing sister-species, sub-species and glacial lineages. Results demonstrate that barcoding is an extremely efficient tool for species identification across all families of North American freshwater fishes and can often identify intra-specific groups. Importantly, our analysis of geographic variation reveals underappreciated phylogeographic breaks shared among different taxa. This knowledge has important conservation implications for those species and further enhances our understanding of the biogeography and evolution of North American freshwater fishes.

BARCODING OF THE FRESHWATER FISH FAUNA OF RUSSIA: A PILOT PROJECT

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In 2006, we started a pilot project "Freshwater fishes of Russia". Our materials have been sequenced at the Biodiversity Institute of Ontario, University of Guelph (c/o Dr. Robert Hanner) being the only project within the Fish Barcode of Life (FISH-BOL) initiative that that aims to assemble a standard reference sequence library for the molecular identification of freshwater and brackish water fishes of Russia. The number of species barcoded is 122 (141 sequences), and we

are close to fix uploading of images and other data including catalogue numbers. Besides Russian fishes, we collected species in continental water bodies all over the Northern Eurasia, e.g. the Caucasus (Georgia and Azerbaijan), the Caspian Sea, basins of the Balkhash and Issyk-kul lakes (Kazakhstan and Kyrgyzstan), drainages of the Black Sea basin in Ukraine and Moldova, and a wide number of samples newly collected was recently included into the project. We follow the ideology of the Barcoding of Life keeping all voucher specimens in a public collection (Zoological Institute, St. Petersburg), and specimen images are available from the Freshwater fishes of Russia web-site (http://www.boldsystems.org/views/projectmenu.php?&).

In the talk, we present some new data concerning phylogeny and phylogeography of some groups of fishes using COI gene and some other genetic markers including discoveries of "sibling" species. Some examples from the most recent studies on complex taxa such as Oreoleuciscus and Rutilus are given to illustrate that the classical morphological and taxonomic data combined with the barcoding data can provide a solid basis for understanding of the taxonomic and faunal diversity of fishes of Russia and neighboring areas.

DNA BARCODE OF THE FISH SPECIES FROM THE SÃO FRANCISCO RIVER BASIN, BRAZIL

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The São Francisco River is the fourth largest river system in South America with a fish fauna composed of at least 152 known neotropical species. Among these, there are several threatened species, some of them endemic from this river basin. Fisheries have evidently declined in recent decades manly because of over fishing, habitat degradation (i.e pollution, hydroelectric dams) and introduction of non-native species. In the 1970s, landings in the São Francisco were around 25 kg/fisherman/day, while in the 1980s they were reduced to about 11 kg/fisherman/day. Accurate and unambiguous identification of fish and fish products, from eggs to adults, is important in many areas. It would enable retail substitutions of species to be detected, assist in managing fisheries for long-term sustainability, and improve ecosystem research and conservation. Therefore, we have undertaken an effort to barcode the ichthyofauna of this system as a contribution to FISH-BOL, the campaign to barcode all fishes. We have already collected and identified specimens from fifty-three species. So far, one hundred and sixty BARCODE sequences have been obtained, representing twenty-six species. All species could be differentiated by their COI sequence, demonstrating that barcoding can achieve unambiguous species recognition for the species analyzed herein. We expect that this data will be an invaluable tool for fisheries managers and fisheries ecologists.

DNA BARCODING OF CUBAN FRESHWATER FISHES: EVIDENCE FOR CRYPTIC SPECIES AND TAXONOMIC CONFLICTS

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Despite ongoing efforts to protect species and ecosystems in Cuba, habitat degradation, overuse, and introduction of alien species have posed serious challenges to native freshwater fish species. In spite of the accumulated knowledge on the systematics of this freshwater ichthyofauna, recent results suggested that we are far from having a complete picture of the Cuban freshwater fish diversity. It is estimated that 40% of freshwater Cuban fish are endemic; however, this number may be even higher. Partial sequences (652 bp) of the mitochondrial gene COI were used to barcode 126 individuals, representing 27 taxonomically recognised species in 17 genera and 10 families. Analysis was based on Kimura's two parameter (K2P) genetic distances, and for four genera a character based analysis (population aggregation analysis) was also used. The mean conspecific, congeneric and confamiliar genetic distances were 0.6%, 9.1% and 20.2%, respectively. Molecular species identification was in concordance with current taxonomical classification in 96.4 % of cases, and based on the neighbour-joining trees, in all but one instance, members of a given genera clustered within the same clade. Within the genus *Gambusia* genetic divergence analysis suggests that there may be at least four cryptic species. In contrast, low genetic divergence and a lack of diagnostic sites suggest that *Rivulus insulaepinorum* may be conspecific with *R. cylindraceus*. Distance and character based analysis were completely concordant, suggesting that they complement species identification. Overall, the results evidenced the usefulness of the DNA barcodes for cataloguing Cuban freshwater fish species and for identifing those groups that deserve further taxonomic attention.

BARCODING FRESHWATER FISHES FROM RIBEIRA DE IGUAPE BASIN, SÃO PAULO - BRAZIL

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The Rio Ribeira de Iguape Basin and the Iguape Estuary Complex, Cananéia and Paranaguá, known as Ribeira Valley, has an area of 28,306 km² within the States of São Paulo and Paraná, Brazil. This area belongs to the Neotropical region, which is the richest in freshwater fish biodiversity of the world. About 100 fish species are recognized for this basin. There are also others with uncertain taxonomic status. The present work proposes to assess the DNA Barcode efficacy to identification of fishes from Ribeira de Iguape Basin. Presently we analyzed about 50% of all species found in this

basin. None of sequences showed insertions, deletions or stop codons. Almost all analyzed species were correctly identified. The average values of K2P distance are 2.05% for species, 8.55% for genus, 12.2% for families. The values presented are similar with that found in others DNA barcode fish works. Thus, we conclude that DNA barcode is an efficient method for species-level identification of the Neotropical freshwater fishes from Ribeira de Iguape Basin.

BIOSURVEILLANCE OF FISH AND ZOOPLANKTON IN NEW ZEALAND LAKES AND RESERVOIRS USING DNA BARCODING

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We tested the utility of DNA-based methods for assessing the diversity of freshwater zooplankton and fish communities. We have trialled 16S rRNA and cytochrome oxidase c subunit 1 (COI) mitochondrial genes and the 28S rRNA nuclear gene. We selected COI as our target region because it successfully discriminated between the species within the groups we have examined (i.e., rotifers, copepods, fish).

This work is being developed with a view to using DNA from environmental samples to enable the rapid and cost-effective screening of aquatic communities (e.g. zooplankton, fish) for conservation management and monitoring of diversity. We have successfully amplified DNA from aquaria water containing eel, goldfish, perch, rudd or trout, and are currently working on optimising procedures to amplify DNA extracted from natural habitats (e.g. water or sediment samples). Trials with zooplankton have been promising, with DNA easily extracted from field samples (e.g. plankton tows). We are also investigating the feasibility of techniques to allow for the automated processing of large numbers of samples cheaply and quickly using a barcoding approach coupled with analysis of length polymorphisms introduced by digestion with restriction enzymes.

DNA BARCODING REVEALS A DISCONTINUOUS GENETIC DIVERSITY PATTERN OF FISH IN THE GODAVARI RIVER, INDIA

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The Godavari river basin, a tropical climate river, is the second largest river in India (1465 km in length). The high level of manmade interference throughout the length of this natural habitat, including dams and hydro-electric plants, makes assessment of fish biodiversity and species

distribution an urgent need for conservation purposes. Up todate information on fish diversity was sparse and relied solely on morphological taxonomy. In this study a comprehensive sampling scheme was applied to represent the fish biodiversity along the full length of this water system followed by analyses of genetic diversity based on DNA sequences of the COI gene.

Fish samples were collected from 8 sampling points covering the geographic distribution of the main river stretch. Fish were taxonomically identified, documented and their fin clips were collected. A 640 bp segment at the 5' of the mtDNA COI gene was amplified and sequenced in both directions. Sequences were aligned, manually checked and then analysed.

Reliable sequence was obtained for 360 specimens representing 39 species, 36 genera in 16 families and 7 orders. The number of variable bases was 299/640 (47%) of which 53% were nonsynonymous substitutions. A total of 99 haplotypes were found that discriminated all 39 taxonomically defined species, with 1-6 haplotypes per species. The phylogenetic tree based on the 99 haplotypes clustered correctly the 39 species into 16 families. In accordance with the expectation from molecular barcoding, molecular variance analysis revealed that 96% of the differences between sequences were due to differences among taxonomic species. Analyses of haplotype sharing and genetic distance were carried out among the three major parts of the river: upper, middle and lower. The lower part of the river had less haplotype sharing with the middle and upper parts. This discontinuous pattern of genetic diversity is probably due to the manmade barriers (dams) separating these regions.

This study demonstrates the usefulness of COI DNA barcoding for studies of biodiversity in inland natural habitats. The results set the reference point to future studies that will monitor the biodiversity status and assess the impact of agricultural and manmade interventions on it for conservation purposes.

DNA BARCODING AND NORTH AMERICAN FRESHWATER FISHES

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Until recent years, morphology has served almost entirely as the criterion and operational tool for identifying species diversity in vertebrates. While neither a requirement in the Code of Zoological Nomenclature nor of most species concepts, diagnoses and descriptions of species have almost universally been based on morphological data. In recent years the international initiative of DNA barcoding has examined the use of a molecular marker, cytochrome c oxidase subunit I (COI), for its potential use in identifying species for a variety of reasons. The gene has also been identified as possessing a greater range of phylogenetic signal than others of the mitochondrial genome at the species level, making it also useful tool for phylogeny reconstruction. Previous studies support the use of the locus on numerous phyla ranging from fruit flies to primates, with a success rate in species identification exceeding 95% of the taxa examined. To explore the usefulness of COI as a

tool for species identification, I have examined the use of COI for several thousand specimens of North American freshwater fishes for species identification and phylogeny reconstruction. My work with Assembling the Tree of Life of Cypriniformes fishes has resulted in a large-scale phylogeny of major lineages in this order. COI, in addition to providing a potentially useful tool for species identification, promises to provide additional important genetic information as to species relationships. I will present the general findings as to both the usefulness of the gene for accurate species identification and for phylogeny reconstruction

DNA BARCODING: REFINING PARATAXONOMY FOR FISHERY SURVEYS

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Parataxonomic sorting of specimens has been considered sufficient approach in biodiversity assessment since it is time-saving. However, it is often associated with sorting errors especially with cryptic species and partially morphologically damaged specimens. In our survey, 664 specimens of freshwater fishes were collected from few rivers and river-mouths in Lake Victoria drainage system in Kenya. The specimens were sorted out into 36 morpho-species using parataxonomic knowledge and further identification was improved by DNA barcoding and Barcode of Life Database System (BOLD). First, digital images of specimens were taken and tissues sliced from muscles and placed in vials with alcohol (95 %). CCDB protocols for DNA extraction, PCR and sequencing was used to generate DNA sequences from the mtDNA COI region. Sequences of length 652 bp were generated. Later, they were edited and aligned in BioEdit version 7.0.0. In BOLD system, Neighbour Joining (NJ) - Kimura 2 Parameter was used to analyze sequences. Sequence divergence within species was 0.11%. 31 haplotypes were revealed suggesting presence of 31 species.

Barcoding confirmed some species which were correctly identified, resolved errors that occurred when specimens were sorted out and recovery of tissues lost due to smudging of taxa ID marks. Within L. Victoria Basin in Kenya, two species of *Synodontis S. victoriae* and *S. afrofischeri* are known to exist. However, DNA barcoding distinguished two specimens from the *S. afrofischeri* taxa they were sorted into due to resemblance in morphology. Distance between their sequences with few *S. victoriae* tested was 4.8 % and *S. afrofischeri* was 7.4 %. DNA barcoding and parataxonomy provided quick solution to species identification but with varying degree of precision and accuracy. In this exercise, results of DNA barcoding provided parataxonomy a means of refining the specimens taxonomic ID and an opportunity to improve on future identification in species inventory.

DNA BARCODE OF FRESHWATER FISHES FROM UPPER PARANA BASIN, BRAZIL

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The Upper Paraná Basin belongs to Neotropical region, which is the richest in freshwater fish biodiversity of the world with about 6,000 species. The Upper Paraná Basin drains a 900.000 km2 area and is inserted in the most exploited region of Brazil. Thus, for example, this region is responsible by 70% of electric production of the country (by hydroelectric power plants). This region has suffered intense human activities, which have strongly changed its natural conditions. About 350 fish species are recognized for this basin. These species represent 10 orders, 38 families and 154 genera. Additionally, we still have many species with uncertain taxonomic status. DNA barcode have shown satisfactory and promising results as a global system for animal and plant identification, including fishes. Thus, the present work is proposed to assess the DNA barcode efficacy to identification of fishes from Upper Paraná River Basin. We obtained sequences from 541 specimens belonging to 90 species, 48 genera, 14 families and 3 orders. No sequences showed insertions, deletions or stop-codons. Almost all analyzed species were correctly identified. The values of K2P distance ranged from 0% to 4.0% (average=0.6%) within species, 1.9% to 19.0% (average=8.7%) within genera, and 12.1% to 21.4% (average=17.6%) within families. Average values for inter-specific K2P distance were 14.5X greater than intra-specific values. The values observed are similar to that found in others DNA barcode fish studies. Thus, we conclude that the DNA barcode methodology is an efficient system to correctly identifying the Neotropical freshwater fishes from Upper Paraná Basin.

DNA BARCODE AND THE HIDDEN DIVERSITY IN THE NEOTROPICAL FRESHWATER FISHES

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The Neotropical Region has about 6000 freshwater fish species. This fauna includes some of the most specious fish groups such as the genera *Astyanax* and *Corydoras*. Many species represent species complexes or cryptic species and some studies have suggested the occurrence of a recent radiation as the main origin of Neotropical freshwater fish fauna. Additionally, the correct species identification is usually very difficult because while some species are wide distributed others are endemics. Considering that DNA barcode has been effective to identify several species complexes and hidden biodiversity in many animal groups the objective of the present investigation is to check the DNA barcode efficacy to indentify species complexes and cryptic species from Upper Parana River Basin in Brazil. We analyzed samples of two species with wide geographic distribution (*Piabina argentea* and *Neoplecostomus paranensis*) and samples of the speciose genus *Astyanax*. Six different clusters were found among *P. argentea* (n=58) and *N. paranensis* (n=47) samples. The K2P genetic divergence among these clusters ranged from 2.0% to 5.6% (average=3.5%), in *P. argentea* and from 1.7% to 8.4% (average=4.9%), in *N. paranensis*. These values are similar with

their congeners species (data not shown). The average K2P distance were 8.8X greater than intra-clusters values in *P. argentea* and 8.9X in *N. paranensis* suggesting the existence of several unidentified new species. Ten recognized species of *Astyanax* were analyzed (n=158). Inter-specific values ranged from 3.3% to 16.8% (average=12.0%). The values were low in many cluster comparisons, which reflects the possible recently radiation of this groups. Thus, for example, the species *A. altiparanae*, recently described, still showed two units with 2.6% K2P divergence between them (4.3X greater than intra-clusters values). Our results show that the DNA barcode is very effective to discover the hidden biodiversity in the Neotropical freshwater fishes.

DNA BARCODING OF COMMERCIALLY IMPORTANT SALMON AND TROUT SPECIES IN NORTH AMERICA

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There is a great need for a reliable system for the identification of commercially important salmon and trout species (*Oncorhynchus* and *Salmo*) in North America, especially one enabling the discrimination of processed material. The present study tests the ability of DNA barcoding to meet this diagnostic need. More than 1000 salmonid reference samples representing eight species were collected from a wide geographic range. DNA extracts from these samples were sequenced for the standard 650 bp barcode region of the cytochrome c oxidase subunit I gene (COI). DNA barcodes showed low intra-species divergences (mean 0.26%, range 0.04-1.09%), and the mean congeneric divergence was 32-fold greater, at 8.22% (range 3.42-12.67%). The minimum interspecies divergence was always greater than the maximum intraspecies divergence, indicating that these species can be reliably differentiated using DNA barcodes. Furthermore, several shorter barcode regions (109-218 bp), termed 'mini-barcodes', were identified in silico that can differentiate all eight species, providing a potential means for species identification in heavily processed products. As the U.S. Food and Drug Administration is currently working towards using DNA barcodes for regulatory species identification, the results of this project will contribute to the detection and prevention of species substitution in commercial salmon products.

GENETIC BARCODING AND THE EVOLUTIONARY ECOLOGY OF EMERGING DISEASES

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Recent analyses of the evolutionary ecology of emerging diseases suggests that changes in climate and geographic distributions can bring susceptible but previously unexposed hosts into contact with pathogens, triggering rapid disease emergence without the evolution of novel genetic information. This means that the current Emerging Infectious Disease Crisis is the tip of the iceberg. If we are not able to become proactive with respect to risk assessment, public health systems around the world will be overwhelmed by many outbreaks, even if they are all relatively minor. This calls for massive international efforts to document pathogen epidemiology in native ecosystems, thoroughly and rapidly. One strategically significant application of genetic bar coding is not affected by the controversy surrounding the use of genetic bar coding in species delimitations. Any given pathogen species exhibits the same genetic barcode throughout its entire epidemiological trajectory through any ecosystem, simple or complex. Genetic barcoding represents a unique opportunity to deal effectively with the Emerging Infectious Disease crisis.

BARCODES OF HELMINTHS OF WILD VERTEBRATES IN MEXICO

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The study of helminths of wild vertebrates in Mexico was traditionally based on morphology. With the use of DNA sequences as an aid for the identification and differentiation of species, an increase in the host and parasite fauna has been discovered as well as the presence of invasive parasite species. Here, several cases of species identification and new species detection are presented using sequences of the mt COI gene: "barcodes".

Barcodes have been useful to identify causative agents of 2 human zoonoses in México. Two new species of the digenean *Paragonimus*, causative agent of the human zoonotic disease "paragonimiasis", were detected in Chiapas and Veracruz. Larvae of the genus *Gnathostoma* can not be properly identified with morphology, but barcodes have been useful for identification of larvae causing creeping migratory disease.

In the case of amphibians and reptiles parasites, 8 new species of the nematode genus *Rhabdias*, parasite of lungs, were detected using barcodes. *Haematoloechus floedae*, a parasite of bullfrogs in the USA, was identified as an invasive species of native leopard frogs in the Yucatán Peninsula and Costa Rica. *Haematoloechus complexus*, a parasite of the lungs of leopard frogs, was revealed to be a species complex containing at least 4 species distributed in México and USA. Also, barcodes

have demonstrated to be useful in the detection of at least 3 undescribed species of amphibian hosts in the Pacific slope of México, revealing a possible co-speciation history of leopard frogs and lung flukes.

MOSQUITO BARCODING INITIATIVE: ANNOUNCING THE FIRST DATA RELEASE PAPER

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The global need to accurately identify vectors of mosquito-borne diseases needs little introduction. Malaria alone claims 1 million lives globally per year. The correct identification of malaria vectors is instrumental in the facilitation of effective vector control. The Mosquito Barcoding Initiative (MBI) aims to produce identification tags for 80% of all the currently recognised World Culicidae by 2010.

Prior to the first MBI data release paper, we present the data from more than 3000 DNA barcodes from species from a wide range of genera, but with a keen focus on the genus Anopheles, some of which transmit malaria. Sequences are all bi-directional, more than 500bp long and generated from vouchered specimens – thus adhering to the red flag barcode standard of GenBank. The MBI have adopted a sytematics approach to barcoding – including specimens from across the range of the species and its type locality where available – to maximise the genetic variation detected. Using DNA sequences from both identified and unidentified species of mosquitoes worldwide to address key questions of this CBOL demonstrator project: Can barcoding help identify species we know? Can it help us uncover new taxa?

Herein we report that DNA barcoding delineates the majority of species tested thus far, and our unique approach to this study discovery of a new genus and 22 new taxa discovered by DNA barcoding in the species barcoded in the genus Anopheles alone, with a high proportion of these detected in the subgenera Nyssorhynchus and Kertezia. Species complexes are common in mosquitoes but we herein show that barcoding rapidly exposes novel taxa. Sceptics of barcoding advocate utility of multiple markers to determine species delineation. Comparison of barcode datasets with thos of the nuclear marker ITS2 have confirmed these as mitochondrial lineages as distinct and novel taxa, but have also highlighted several cases of mitochondrial introgression in

members of the Pyretophorus Series. Preferential amplification of vertebrate hosts using the universal barcode primers of Folmar et al. (1994) also occurs.

BARCODING ADVANCES FRESHWATER FISH PARASITOLOGY

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Larval helminths are among the most common parasites of freshwater fish and cannot be identified to species based on morphology. Larval trematodes of the family Diplostomoidea (Platyhelminthes) are a particularly widespread cause of pathology in fish and can negatively affect aquaculture. We used barcode-region sequences of cytochrome oxidase I to distinguish species of larval diplostomoids from nearly 1000 fishes in the St. Lawrence River, Quebec, Canada, with stratified replication of host taxa and sampling localities. Corroborating sequences from internal transcribed spacer regions of rDNA were obtained for most species, but interspecific resolution was less clear with these more commonly used markers. In fewer than half the fish species present in a single river, four times as many species of diplostomoid parasites were detected by barcoding than have been recorded in all freshwater fishes in Canada, where fish parasites have been relatively well studied. Barcodes revealed unanticipated patterns of host specificity based on the site of infection in the fish. We found that most larval diplostomoids are host specific, but species that infect the lenses of the eyes of fishes infect a range of hosts that is significantly larger and more diverse. We suggest the limited immune response in the lens is what allows lens-inhabiting parasites to colonize more host taxa. The stratified sampling design and barcode-identifications allowed a robust evaluation of factors that affect parasite community structure. We assessed whether parasite communities are best considered attributes of host taxa or of habitats and found that, within our system, host phylogeny is much more important in structuring parasite communities than spatial scale or habitat effects.

DO WE NEED BARCODES FOR THE PARASITIC HELMINTHS?

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Parasitic helminths often present a multitude of taxonomic conundrums because of their small size, soft bodies and morphologically indistinct larval stages. The application of molecular biology to parasite taxonomy and systematics is widespread, but the molecular tools applied vary among taxa, with ITS being used most often for trematodes and nematodes, and 18S and 28S for cestodes. Barcodes have been rarely used, but to date they have been very successful in discriminating among strigeid trematodes, a notoriously problematic group taxonomically. Many diseases of fish and wildlife, both established and emerging, are caused by parasitic helminths.

However, to identify the species of the pathogen, one must kill the host, and in some cases only male helminths are identifiable. Practical tools for species-identification based on females and, in particular, transmission stages in host excreta, would therefore be highly desirable. In addition, pathology can be caused by larval parasites, which cannot be identified to species morphologically even when removed from the host. Thus, ecological and epidemiological studies become difficult to implement, and results may be confounded by poor taxonomic resolution. Examples include anisakid nematodes of marine fish, eyeflukes in freshwater fish, and echinostome trematodes in frogs. The extensive ecological work done on these parasites to date may require re-evaluation or at the very least cautious re-interpretation. We will present data from various ecological datasets, many of them from the senior author's previously published works, using recently-derived knowledge of parasite taxonomy, to highlight the importance of good taxonomic resolution in ecological and epidemiological studies. The consequences of these taxonomic developments will be explored for the design of future studies, especially those involving emerging diseases. We will conclude by evaluating the potential role of barcodes in disease ecology.

NEMATODE DISEASES: THE PLURALITY OF DNA BARCODING

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Nematode parasites of vertebrates are one of the most lethal agents of diseases that affect crops, humans and livestock. Of these, only those that belong into the Ascaridida (16), Spirurida (611), and Oxyurida (14) add up to 641. This does not only mean that there is a shortage of sequences for vertebrate parasitic nematodes, but shows that what is probably the oldest known group of metazoan parasites is surprisingly underrepresented by DNA barcodes. Free-living soil nematodes have been successfully barcoded with the SSU of the rRNA. This might indicate that other molecular markers arguably rank as good as COI, yet their power of discrimination is debatable. In the case of nematode parasites of vertebrates while COI barcoding seemingly has been successful, SSU seems to be not as successful for species differentiation.

Several problems can be identified with parasitic nematode barcoding. The main one is that primer design for COI is no easy task. Primer pairs work very specifically and their utility cannot be extended to other nematode species, even within the same genus. Therefore, longer COI sequences or even the complete mtDNA need to be known before hand and these should include the barcoded region desired. Species discrimination in a barcoding fashion can be achieved through the usage of nuclear markers such as 28S. A renewed strategy for barcoding vertebrate nematode parasites massively and in a short period should include not only one marker, but at least a couple of nuclear markers with several tandem repeats, such as the one mentioned and probably ITS1 and ITS2.

POTHOLES IN THE ROAD TO DNA BARCODES FOR PARASITES

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A quick, easy, and accurate method of identification of organisms in the field and laboratory that is available to everyone seems like something that everyone would favor. However, even though the data collection process necessary for deriving a DNA barcode is not often openly opposed, there appear to be many that still remain skeptical, and some have challenged the need for it in published works. Although some parasitologists actively are participating in the endeavor, the skepticism of parasitologists as a group surpasses that of those in other areas of investigation.

The purpose of this presentation is to consider DNA barcoding from the viewpoint of morphologists as a whole (those more likely to oppose molecular studies), and parasitologists in particular, and to identify possible objections to this line of investigation, some of which have appeared in publications.

Objections to and/or dissatisfaction with what has become known as a "DNA barcode" for all species fall into 3 general categories. In the first category are objections caused by a misunderstanding of, or lack of, understanding of what the barcoding process is and what it promises to provide. These objections may be related to theoretical or methodological issues, but all potentially can be countered by education in various themes. Other objections concern theoretical issues related to delimiting species or how the data should, or could, be used. A large proportion of objections are similar to those raised concerning any molecular study: how to collect DNA, the appropriate genes to use, and how to analyze the data. However, some are methodological questions related intrinsically to parasitology: difficulty in collecting parasites, degree of genetic information shared with host species, problems of scale, and differences related to the parasitic lifestyle. These objections, possible remedies, and potential benefits of universal barcodes for parasites are discussed.

USE OF DNA BARCODING TO DETECT INVASIVE SPECIES AND SOLVE TAXONOMIC PROBLEMS WITHIN HIRUDINEA

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The use of short sequences of DNA to identify leeches has been applied in the last 10 years, typically with good success. Recently, it has become clear that in certain groups the use of a single sequence of mitochondrial DNA (e.g., COI) is potentially risky, for instance in some species of *Erpobdella* in Mexico the intraspecific variation of COI sequences can exceed the variation between species that are recognized by taxonomists. Nonetheless, and in terms of species

identification, DNA barcoding has proven useful in detecting globally invasive species, whereas in terms of species delimitation, barcoding has been used to identify a variety of species. The true distribution of *Barbronia weberi*, a presumptive native of the Indo-Pacific region, and the complex taxonomic and biogeographic distribution of a South American species of *Helobdella* are examined with DNA barcoding. In addition, we present the redefinition of a group of leeches that parasitiseze a wide variety of vertebrates, including humans. This analysis ranges from the taxonomic identification using DNA to the phylogenetic analysis of the family Praobdellidae and can be useful to exemplify the use of barcodes as the initial step toward a more deep taxonomic revision of leech taxonomy with implications on human health.

GENETIC STUDIES OF CHYTRIDIOMYCOSIS, AN EMERGING INFECTIOUS DISEASE OF AMPHIBIANS

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Amphibians are declining worldwide at an unprecedented rate, resulting in loss of biodiversity and serious consequences for ecosystems in which they play critical roles. Habitat loss, climate change, and emerging diseases contribute to these amphibian extinctions. The chytrid fungus Batrachochytrium dendrobatidis (Bd) causes chytridiomycosis, a disease that has been implicated in amphibian decline worldwide. Bd causes mortality in most species, but some are apparently resistant to the disease and thus may serve as reservoirs or vectors for movement of the pathogen to new populations. Epizootic events in wild populations can result from outbreaks of endemic pathogens, arrival of novel pathogens, or novel mutations within a pathogen's genome. Based on these mechanisms, a number of competing hypotheses have been proposed to account for outbreaks of Bd in the Neotropics. Among these, the spreading pathogen hypothesis (SPH) proposes that the spatiotemporal spread of Bd into new regions and naïve host populations occurs through natural or human-mediated dispersal. A recent review of Bd-induced population declines in Neotropical frogs provides some evidence in support of the SPH in Central America and identifies the most likely potential introduction sites and epidemic waves in the Andes of South America; however, this hypothesis needs to be tested with analyses of the population dynamics of the pathogen itself.

The use of a genetic bar coding for the study of Chytrid fungus in different regions of the world, but especifically in MesoAmerica where the pattern of a temporal wave has been documented will help to quantify population genetic patterns that reveal the history of Bd in this area. Under the SPH, we expect genetic signatures of population expansion at the leading edge of each wave due to sequential bottlenecks, which purify the population of genetic variants during epidemic spread. A comparative investigation of selected 'pathogenicity' gene diversity between and within different geographic Bd fronts will contribute to our understanding of the rates of evolution within these populations, and allow us to test for an association between genetic diversity and patterns of spatiotemporal spread.

DNA BARCODING HIGHLIGHTS ISSUES WITH MORPHOLOGY-BASED TAXONOMY OF NEOTROPICAL BLACK FLIES (DIPTERA: SIMULIIDAE)

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Black flies are a worldwide family of nematocerous Diptera with 2072 formally named species. At least 350 nominal species are known from the Neotropical region, including vectors of Human Onchocerciasis. In this study, we analyzed 1040 specimens of black flies collected from Brazil, Venezuela, Peru, Ecuador and Bolivia to test the utility of the DNA barcoding gene for discriminating species from the Neotropical region. Seventy species have been barcoded to date, representing about a fifth of all known Neotropical species. Mean intraspecific genetic divergence for well-established species was 1.08%. High levels of intraspecific variation was found among widely species; for example, specimens of Simulium lutzianum collected from Brazil, Ecuador and Venezuela had a mean divergence of 6.24%, indicating that this 'species' might actually consist of a number of isomorphic sibling species. Statistical analyses compared genetic and geographic distance matrices of 16 populations of Simulium subnigrum, revealing that genetic variation is correlated with geographical distance between populations. In general, DNA barcoding proved effective for discriminating most Neotropical species. The approach also revealed the presence of 3, previously unrecognized, sibling species. A multifaceted approach incorporating morphological-cytological- and DNA sequence data is critical for addressing species-recognition problems in certain problematic lineages.

MOLECULAR PROSPECTING FOR CRYPTIC SPECIES OF PARASITES: ARE DNA BARCODES USEFUL?

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The discovery of cryptic species in nature (morphologically indistinguishable, genetically distinct), has attracted the attention of systematists, ecologists and evolutionary biologists because such species have significant implications for evolutionary theory, biogeography and conservation planning. Parasitologists discover and describe new species of parasites with regularity, and DNA-based taxonomic methods are increasingly used to complement these descriptions. Implicit in their discoveries is the possibility to find cryptic species. I briefly review problems with recognizing cryptic species of parasites and I show a distinction between cryptic species prospecting (methods to detect putative cryptic species) and delimitation (testing we have them).In some animals, it has been shown that by using short DNA sequence of a standardized gene region, specimens can be

assigned to species, but also that cryptic species might be detected. Potential uses of DNA barcoding have been challenged; two uses are often confused—species identification and species discovery. Species identification is unequivocally a valid use of DNA barcoding, since sequences are used as markers for a priori established species. Species discovery cannot solely use DNA barcodes as the arbiter of the discovery of new species. Similarly, detection of cryptic species might be a valid use of DNA barcoding, but the hypothesis testing for delimiting such species requires further consideration. I also evaluate the potential of DNA barcoding on detecting cryptic species of parasites. It is important for obtaining an accurate inventory of extant biodiversity, and failure to recognize cryptic species in medically, economically or ecologically important parasites can have serious consequences for the development of biological control measures, monitoring and control of human diseases and potential zoonoses, management of agricultural and aquaculture pathogens, and detecting the presence of invasive species.

DNA BARCODING OF MOSQUITOES IN INDIA

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Mosquitoes are very important group of insects, many of which act as vectors for malaria, filariasis, dengue and chikungunya fevers, japanese encephalitis etc, the major vector borne diseases affecting millions in India.

India has a reported biodiversity of about 350 species of mosquitoes (20 Genera). Species identification of mosquitoes currently carried out based on morphological characteristics pose many difficulties in identification of species involved in endemic/epidemic disease situations, crucial for an effective disease management strategy.

Our preliminary studies on DNA Barcoding of mosquitoes evidenced the utility of this tool for species identification even from a very small portion of the specimen (Pradeep Kumar *et al*., 2007). Hence, we initiated a project on DNA Barcoding of all the mosquito species present in India, as a complementary approach to conventional taxonomic tools, aimed at development of a database towards a comprehensive system for efficient species identification of these insects. Specimens were collected from different eco-geographical zones; morphologically identified; voucher specimens preserved and 2-3 legs of the specimen are processed for DNA Barcoding.

So far, 503 specimens of mosquitoes collected from 15 States/UTs in the Country which belonged to 143 morphologically identified species (18 Genera) were processed. DNA Barcode approach identified all these except one (*Aedes portonovoensis*) as individual species. Besides, few morphological species (*Armigeres subalbatus*; *Verallina* sp.) were found to be complexes of cryptic species. Major sibling species complex members of Anphelines viz., *Anopheles culicifacies*, *An.*

subpictus could be characterized by this approach. The studies are ongoing so as to develop DNA Barcodes for all the mosquito species prevalent in India.

Technical Session Abstracts – Tuesday

Plant Working Group

USE OF ITS2 REGION AS UNIVERSAL BARCODE TO IDENTIFY MEDICINAL PLANTS AND THEIR ADULTERANTS

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A DNA barcode is a short DNA fragment from an organism's genome that can be used to identify a species. Until today, no standard regions have been identified to discriminate plant species in the same way as the coding region for cytochrome c oxidase subunit 1 (COI) gene in animal species. In this study, we tested seven candidate DNA barcodes for plant species used in traditional herbal medicines. Our ranking criteria include PCR amplification efficiency, differential intra- and inter-specific divergences, and DNA barcoding gap. Our data suggest that the second internal transcribed spacer region (ITS2) of nuclear DNA represents the most suitable region for DNA barcoding applications while the internal transcribed spacer (ITS) fails to be a standard barcode due to its lower efficiency of PCR amplification. Furthermore, through analysis of >6600 plant samples belonging to 4800 species based on BLAST and the nearest distance methods, the rates of successful identification with ITS2 were >90 % at species level. In comparison, the psbA-trnH spacer region showed >70 % success rate at species level. In conclusion, our study suggests that ITS2 locus can potentially be used as the standard barcode for authenticating medicinal plant species used in traditional herbal medicines and their adulterants as well as all major plant taxa.

BARCODING THE WOODY ANGIOSPERM CLADE VIBURNUM

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Recent studies have promoted the use of portions of the chloroplast genes *rbcL* and *matK* for DNA barcoding in green plants. These studies have evaluated potential barcoding markers based on universality, sequence quality, and species discrimination. Species discrimination has been judged using two or more species of unspecified relatedness from a diverse array of genera. While this approach has been useful, it does not adequately evaluate the ability of candidate markers to discriminate among closely related species. In this study we have taken an alternative approach, focusing on comprehensive sampling within a single clade of woody angiosperms, *Viburnum*, with some 160 species in temperate and montane forests primarily around the Northern Hemisphere. *Viburnum* is of great economic value to the horticultural industry, is routinely moved across international borders, and may become invasive; therefore, it would be highly beneficial to

accurately differentiate species using DNA barcodes. We sampled over 100 species of *Viburnum* and screened *rbcL* and *matK* in addition to *trnH-psbA*, *rpl32-trnL*, *trnK*, and ITS. We report identification success for *Viburnum* species based on genetic distances and neighbor-joining trees for all combinations of gene regions sampled. In addition, given the potential use of barcoding in a regional context, we analyzed *Viburnum* data from three areas: Central America, Ecuador, and Japan. Although these markers do consistently identify previously recognized species groups ("sections"), they very often fail to discriminate among closely related species within these clades owing to a lack of variation. We suspect that results of this sort will be obtained in many other woody plants, where rates of molecular evolution may generally be slower, perhaps owing to longer generation times. These disappointing results highlight the need to explore alternative markers, and we present evidence on the utility of an intron of the nuclear gene *leafy*.

LEAVES OF GRASS: BARCODING AN ICONIC AND ENIGMATIC PLANT FAMILY

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The grasses (family Poaceae) constitute one of the most largest families of plants in the world. They contain our most important grain crops, including wheat, rice, and maize, and by contrast, they also comprise many of the world's most problematic invasive species. Grasses form a critical component of many ecosystems and their adaptability to extremes of heat and aridity make them excellent candidates for functional genetic research. Our group has begun the Grass Barcoding of Life project (GrassBoL) to unite grass researchers around the world in DNA barcoding all species of the family, develop additional 'local' barcodes, and explore possible practical and pure research applications. This project will span many disciplines, including taxonomy, systematics, forensics, ancient DNA, agronomy, genomics, ecology, invasive species research and archaeology. In the first phase of this project we are testing the plant DNA barcoding markers in representatives from all major subfamilies of grasses in Australia, with greater representation and focus on selected genera. We have also tested several additional DNA markers on targeted genera of closely related species and those for which the species boundaries are uncertain. We have found that the plant barcoding markers rbcL and matK work well to distinguish among larger, more widespread genera, but not as well for more closely related taxa which have probably radiated more recently. Our trials using a range of markers have met with some promise, but have also demonstrated some limitations. We will discuss the results and patterns we have observed across the family using our initial work applying these markers towards research in ecology and adaptation of the Poaceae. Because of their ecological importance, this project will have broader implications for research on monitoring ecosystems with the goal of defining a plant community by the presence and absence of specific species, as well as the effect of invasive grasses

PATTERNS OF PLANT SPECIES DIVERSITY BELOW GROUND AS REVEALED BY DNA BARCODING

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Plant DNA barcode regions from the plastid genome have been identified and tested for their use in discriminating species. However, the applications of barcoding to ecological studies are largely unexplored. Here, we tested the effectiveness of one plant DNA barcode region for investigating patterns of below ground plant diversity and its determinants, through an analysis of roots. A total of 3800 roots were collected from four randomly positioned soil profiles located in an old field community. More than 1500 roots were sampled for DNA extraction and sequenced for a portion of the plastid gene rbcL. Species were identified by comparing root sequences to a sequence library established using the above ground flora. Nearly 85% of sampled roots were successfully identified as belonging to 28 different plant taxa. Root abundance and species diversity were negatively correlated with soil depth. However, individual species had different root profiles, possibly reflecting different root architectures and life histories. Root abundance did not always correspond to taxon frequency above ground and some species exhibited non-random patterns of co-existence. Ordinations of above ground and below ground data indicate contrasting patterns of community structure. Our results show that barcoding can provide important insights into the organization of diversity below ground and the illuminate the potential role of factors such as competition and phylogeny.

GENEALOGICAL NONMONOPHYLY IN *PINUS* AND ITS RELEVANCE FOR DNA BARCODING

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Pinus (Pinaceae) is an ecologically and economically important genus comprised of approximately 115 species occuring naturally throughout the northern hemisphere. The genus is divided into two subgenera, *Strobus* (the soft pines) and *Pinus* (the hard pines), four sections, and at least 11 subsections. Absolute age estimates using relaxed molecular clocks suggest that while the subgenera are relatively old (Late Cretaceous stem age), the crown ages of subsections are young, mostly dating to the Miocene. Sequences from the plastid coding regions *matK* and *rbcL* have been used to place species in one of 11 subsections, but *matK* and *rbcL* together are not always variable enough to distinguish among species within these clades. The problem is most acute in two subsections of North American hard pines, *Ponderosae* and *Australes*. These subsections rank as the youngest and most diverse; the approximately 17 species of subsection *Ponderosae* and the approximately 29 species of subsection *Australes* are estimated to have diversified in the last 10

million years (probably in the Miocene, possibly in the Pliocene for subsection *Ponderosae*). Shorter reads from *matK* and *rbcL* have been proposed as the plant DNA barcode markers. Based on sequence variation in three individuals per species in the sections *Australes*, *Ponderosae*, *Cembroides*, and *Strobus*, probably fewer than 50% of sister species will be descriminated with the barcode markers, particularly those in young, species rich clades. A possible solution for low species descrimination is to include additional loci. However, a growing number of studies in *Pinus* are documenting that incomplete lineage sorting and interspecific gene flow have influenced the partitioning of nuclear and plastid DNA between and within species. Incomplete lineage sorting in pines probably has been promoted by high outcrossing rates via wind pollination, long, overlapping generation times, and large effective population sizes. Interspecific gene flow probably has been facilitated by allopatric speciation in the absence of intrinsic reproductive barriers, followed by secondary contact. Genealogical nonmonophyly may pose an important limitation of using DNA barcoding in long-lived perennials with large effective population sizes.

SEEING THE FOREST FROM THE TREES: AUSTRALIAN TREE DIVERSITY

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Trees define tropical rainforests, and the diversity of tree species in these ecosystems is very high. However, more work is needed regarding how species diversity changes between different forest habitats and how the distributions of these species vary throughout a region. Still less is known about the impact of different foresty and timber practices on rainforest diversity. Phylogenetic Diversity (PD) analysis is a method whereby the molecular phylogeny of species in an area is used as a measure of biodiversity. We have conducted PD analyses on trees from several rainforest plots in the World Heritage listed Wet Tropics located in northeastern Australia utilizing the plant DNA barcoding markers rbcL and matK. Our results provide a novel, taxonomic rank-independent picture of comparative diversity across rainforest plots that is much more detailed than has been possible to date. The DNA barcoding loci provide a standardised marker set for land plants potentially allowing comparative biodiversity assessments across all ecosystems - a truly global perspective on biodiversity. Our work has also highlighted the utility of mini barcodes designed for sequencing of degraded wood material. These techniques will have many applications, including the potential of tracking timber products and monitoring forest usage in many ecosystems. Our research shows the utility of DNA barcoding for studies in biodiversity, ecology, and good forest management practices focused on monitoring illegal logging.

DNA BARCODING IN THE MEXICAN CYCADS USING CAOS

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A DNA barcoding study was conducted to determine the optimal combination of loci needed for successful species-level molecular identification in three extant cycad genera, Ceratozamia, Dioon and Zamia that occur in Mexico. Based on conclusions of a previous study in representative species of all genera in the Cycadales, we tested the DNA barcoding performance of six chloroplast coding (matK, rpoB, rpoC1) and non-coding regions (atpF/H, psbK/I, trnH-psbA), plus sequences of the nuclear ITS. We analyzed data under the assumptions of the "Character Attributes Organization System" (CAOS), a character-based approach whereby species are identified through the presence or 'DNA diagnostics'. In Ceratozamia, five chloroplast regions were needed to achieve >70 percent of unique species identification, whereas the two-gene atpF/H+psbK/I and the four-gene combination atpF/H+psbK/I+rpoC1+ITS2 were needed to reach 79% and 72% of unique species identification in Dioon and Zamia, respectively. The combinations atpF/H+psbK/I and atpF/H+psbK/I+rpoC1+ITS2 include loci previously considered by the international DNA barcoding community. However, our results suggest that the optimal combination for DNA barcoding in cycads might not coincide with the 'core barcode' of chloroplast markers (matK +rbcL) recently proposed for universal use in the plant kingdom.

PLANT BARCODING IN TAXONOMICALLY COMPLEX GROUPS: GRASSES AND WILLOWS

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The grasses (Poaceae) and willows (Salicaceae) are plant families that provide many challenges to species identification. Taxonomic concepts in both groups have been fluid and continue to undergo systematic revision. Both are ecologically important, and in each case members have been used as ornamentals or classified as invasive weeds. What role can plant barcoding play in species identification in these complex groups? We assess the discrimination performance of the two regions proposed for plant barcoding (*mat*K and *rbc*L), in addition to other coding and non-coding regions (*atp*F-*atp*H, *psb*K-*psb*I, *rpo*B, *rpo*C1, *trn*H-*psb*A). Most loci were unproblematic to amplify but there was some variation in sequencing success between regions. Our taxonomic

sampling includes, in most cases, multiple close relatives, and multiple populations per species, mainly sampled from the Canadian province of British Columbia, but also more broadly across North America. We also focus on a number of complex grass genera (*Bromus*, *Calamagrostis*, *Festuca* and *Poa*) and the complex willow genus *Salix*.

PLANT DNA BARCODING AND CONCEPT OF A UNIVERSAL LOCUS MAY NOT WORK IN COMPLEX GROUP: A CASE STUDY WITH *BERBERIS*

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We investigated the species discriminatory power of four plant DNA barcoding loci (one from nuclear genome- ITS, and three from chloroplast genome- *trnH-psbA, rbcL* and *matK*) in genus *Berberis* L., the largest genus of the family, *Berberidaceae* and two other well defined genera *viz.* *Ficus* L (Moraceae) and *Gossypium* L. (Malvaceae). We analyzed multiple accessions for all the species. PCR success ranged from 76% to 100% across the three genera for the four loci. Sequencing success ranged from 85% for *matK*-NBRI (modified *matK*) to 100% for ITS. In Wilcoxon sign ranked test, ITS emerged as the most divergent barcode locus at interspecific level. In *Berberis,* we did not find any barcoding gap corresponding to inter- and intra- specific variability in any of the four loci. However, ITS locus showed barcoding gap in *Ficus* and *Gossypium.* Analysis of species recovery using NJ, MP and UPGMA methods for single locus as well as multilocus combinations showed that none of the loci was able to discriminate species of *Berberis,* In multilocus analysis, two loci combinations of ITS and *trnH-psbA* provided better species resolution (30.77%) as compared to the best single locus (6.25% with ITS). 100% species recovery was observed using ITS and *trnH-psbA* in *Ficus.* ITS recovered 100% species in *Gossypium*. Other loci were not able to differentiate the species of *Ficus* and *Gossypium.* Applying nucleotide character-based approach for species identification, no diagnostic character was found in any of the loci in *Berberis* to distinguish its species except in *B. pachyacanha*. However, there are well defined nucleotide characters to distinguish species in *Ficus* and *Gossypium*. Morphometric analysis using twenty five characters resolved the *Berberis* species well. Barcoding in genus like *Berberis* is challenging because of the occurrence of natural hybrids. Our results with *Ficus* and *Gossypium* suggest that ITS and *trnH-psbA* are good candidates for plant DNA barcoding. The utility of *matK* as universal plant barcoding locus needs re-evaluation.

UTILTIY OF PLASTID "BARCODES" TO IDENTIFY PLANT SPECIES

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The recommendation on a standard plant barcode provides a first step towards a wide application of barcoding for plants. The next major challenge is the need to convince botanists and other users that these barcodes will provide unambiguous identification for the majority of extant plant species. Many botanists expressed doubts about the capabilities of plastid genome based barcodes to identity plants as a result of the large impact of agamic complexes and hyrbidization on the psyche of plant taxonomists. We explore the utility of existing and newly generated sequences of selected plastid genome regions to distinguish more than 30 closely related species belonging to various fern genera such as Alsophila, Asplenium, Lepisorus, Phegopteris, and Pleopeltis. Ferns are expected to provide obstacles to DNA-based identification because they are well known for hybridization and polyploidization. At the same time, research on fern ecology will highly benefit from barcoding protocols allowing the identification of both free-living generations. Morphology is usually only suitable to identify the sporophyte, the larger of the two free-living generations, whereas the dimidiate gametophyte is ignored. Our test cases include lineages that diversified in the last 4-5 million year.   Plastid genome based barcodes generated unambiguous identification for all case groups as long as only diploid taxa are considered with the exception of tree ferns (Alsophila). Our analyses confirmed the need to introduce new species that are in agreement with other available data. In conclusion, our results support the promise of DNA-based identification tools to plant research as long as we accept limited utility to identify apomicts, hybrids and recent polyploids. Despite these are important limitations; only a small fragment of plant diversity represents these issues, e.g. less than 1% of all land plants are reported to be apomicts.

AN EVALUATION OF MULTILOCUS DNA BARCODES IN FIVE MEXICAN PLANT GROUPS

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Five groups of Mexican vascular plants corresponding to eight families were selected for DNA barcoding.   Representative taxa in Agavaceae, Cactaceae, Crassulaceae, Orchidaceae, and Coniferales (Cupressaceae, Pinaceae, Podocarpaceae y Taxaceae) were chosen. These groups are crucial because they are either endangered, dominant in habitats, amply utilized or diversified mostly in Mexico. We evaluated the plastid coding regions *matK* and *rbcL* and the intergenic spacers *trnH-psbA, psbK-psbI, trnL-F*, *rpL32-trnL* and *trnS-G* . The *matK* and *rbcL* loci were recently selected for barcoding the plant kingdom, but complementary sequence information may be

necessary for groups with low levels of molecular divergence. Approximately 177 species (38 from Agavaceae, 24 from Coniferales, 28 from Cactaceae, 8 from Crassulaceae and 79 from Orchidaceae) were sequenced. The spacers *trnH-psbA* and *trnG-trnS* were problematic because they did not amplify in all groups, moreover *trnH-psbA* with less than 400 pb in Cactaceae was not variable.

COMPILING AND EXPLOITING A NATIONAL BARCODE FOR WALES

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The nation of Wales covers 20,779 Km^2 and ranges in altitude from sea level to 1085m. Its flora, which is one of the best characterised in the world, contains approximately 1200 native/archeophyte species of higher plants. The entire landscape of Wales has been geophysically characterised down to a resolution of <25m^2, phase 1 habitat classification to a level of 1km2 and 2-10km2 resolution distribution data for all flowering plant species. In this presentation, we will first describe the process of assembling and double-verifying voucher specimens of all native/archeophyte species of flowering plant in Wales, outline problems associated with DNA extraction from fresh and herbarium material, and with the generation of a comprehensive sequence data set for matK and rbcL. We then go on to outline how it is possible to increase resolution and practical utility of genetic barcode by reference to spatially explicit community species assemblages. Finally, we will provide two exemplars of how a comprehensive geographic set of barcodes can be used for landscape scale ecological research. The first study combines total community barcodes with second generation sequencing to characterise differences in pollinator behaviour and to provide a community level assessment of pollination as an ecosystem service. The second study exploits pyrosequencing approaches to compare differences in long-range pollen carriage between pollinators of crop species and interpret our findings in the context of GM crop risk assessment.

Algae, Fungi, Protists & New Groups

BIOCODING THE FUNGI OF MOOREA

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The Moorea Biocode Project is attempting to characterize, through biological collections and DNA "barcodes," all non-microbial life on the island of Moorea (French Polynesia) and in its surrounding coral reefs. The island, although tropical in climate and characterized by rich and complex ecosystems, is relatively young in origin and is known to harbor relatively simplified communities because of its geographic isolation. This combination of factors make it an ideal site to attempt a complete biological inventory. Fungi are the only microbes currently included, partially because this kingdom includes both macro- and micro-organisms. Goals of the fungal survey include: a) Conduct a baseline diversity assessment for macrofungi; b) Compare endophytic fungal diversity between several of the most common Moorea tree species; c) Isolate, describe, and barcode all plant pathogens causing visible symptoms. Because of the expected overall high diversity of the fungal community and the difficulty of culturing some groups of fungi, a classical biological inventory is being augmented by environmental DNA sampling to allow for an estimation of real fungal diversity and species composition. Both lines of research are being conducted across a land-use gradient ranging from pineapple plantations to Polynesian abandoned agroforestry to endemic plant communities on mountain ridgetops. Barcoding is being conducted using the ITS locus with the addition of the 5' portion of the more conserved 28S in the ribosomal RNA operon. This ongoing effort is providing some of the most complete information to date of actual fungal biodiversity, a highly discussed topic, while developing and testing protocols for direct environmental sampling. Results so far point to rather diverse communities dominated by potentially novel species. An evaluation of possible biases and shortcomings of direct environmental sampling and an overall discussion of the fungal diversity so far captured will be presented.

THE FRAMEWORK TO BARCODE NEOTROPICAL ECTOMYCORRHIZAL FUNGI

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It is estimated that there are more than 50000 species of ectomycorrhizal (ECM) fungi. Somewhat 7% have been described based on their reproductive fruit bodies. Studies on the molecular ecology of ectomycorrhizae have shown that they are a high diverse group with up to 300 species in a single forest. Sequences of ectomycorrhizae commonly belong to unknown taxa. Many taxa do not have sexual reproduction. Additionally the number of taxonomists in these groups is scarce. The resulting scheme is an hyperdiverse group with a poor understanding of its taxonomy. In

underexplored areas as the Mexican Neotropics, where no more than five monographies on ECM fungi have been published, the lack of this knowledge is a common barrier to the development of science. Barcoding ECM fungi is a promissory tool to partially solve this problem. However, given the biology, ecology and the state of the art of ECM fungi taxonomy, a series of considerations should be taken into account.

Here we discuss the framework, techniques and preliminary results of a project devoted to barcode the ECM fungi in Mexican Neotropics. To provide accurate taxonomic identifications we decided to get support of available taxonomists inside and outside Mexico. Materials not corresponding to known species are named as "affine" to the closest described taxa or as unknown species within sections or subgenera. With this strategy we by pass the "taxonomic problem" and make available to the scientific community specimens and sequences that otherwise would be stored for years. Given that a considerable amount of ECM fungi do not produce sexual fruit bodies we decided to produce barcodes of vegetative structures (mycorrhizae). Although mycorrhizae are considered environmental samples, many fungi -former Deuteromycota- are indeed classified using their asexual stage and some groups as Penicillium already are been barcoded.

BIOCODING THE FUNGI OF MOOREA

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environmental sampling. Results so far point to rather diverse communities dominated by potentially novel species. An evaluation of possible biases and shortcomings of direct environmental sampling and an overall discussion of the fungal diversity so far captured will be presented.

BARCODING OF MEDICAL FUNGI - ITS REGION AND ITS LIMITATIONS

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Invasive fungal infections (IFIs) are on the rise due to an ever-increasing number of immunocompromised and otherwise debilitated patients and the emergence of new fungal pathogens. Management of IFIs is problematic since current identification techniques are insufficient, therapies are limited in efficacy and/or safety, and resistant fungi are emerging. Targeted intervention strategies that hinge on accurate and early identification are required to improve patient outcomes. Classical identification (morphology, physiology) is slow and often incorrect.

Sequence based identification strategies are the new "gold standard" for species ID. The Internal Transcribed Spacer (ITS) regions of the ribosomal DNA gene cluster has been selected as default for fungal barcoding and is now widely used as an alternative to classical identification in mecial mycology. However, beside the growing number of ITS databases there are currently no ITS sequences of medical relevant fungal species deposited in the BOLD identification system.

Sequenced based ID in the medical field is currently based on a cut-off of 98-99% similarity with the type culture of the species in question. Population based studies of 480 strains representing 182 human fungal pathogens have shown that the sequences variation in clinical samples is much higher as those type culture dependent cut-off values. The results show that fungi have species dependent variable rates of polymorphisms in their ITS1/2 regions. Intra-species variation varied from 0 to 8.35%, with C. parapsilosis showing 0% and C. tropicalis having as much as 8.35%.

A quality controlled ITS database is now available for comparative sequence based fungal ID at: http://www.mycologylab.org/BioloMICSID.aspx. These sequences offer on one side a valuable addition to the BOLD system by filling the gap concerning human pathogenic fungi, but also raise on the other side the question if the ITS region is the most appropriate locus for fungal barcoding.

BARCODING INVASIVES: A NEW TOOL FOR INVASION MONITORING IN SOIL

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Biological invasions are gaining substantial attention from policies makers. All biotas are—or are about to become—impacted by global changes and the soil fauna is no exception. However, the difficulties inherent in taxonomic surveys of the soil-dwelling organisms hamper the monitoring of this ecosystem compartment. Collembolans and earthworms are two key groups of the soil fauna that have been targeted for barcode analysis. Ten earthworm species and 6 collembolan species were used to test the efficiency of barcoding to monitor biological invasions in soil: European invasive earthworms in North America and European invasive collembolans in North America and Oceania. Present results establish the usefulness of DNA barcoding for the quick and low cost surveys of soil biodiversity.

BARCODING ORTHALICID LAND SNAILS FROM PERU

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Orthalicidae is the most representative family of land snails in the Peruvian desert coast. However, the diversity of this group remains unknown and its classification is controversial. Genomic information especially DNA barcodes has become a key factor to understand evolutionary relationships and to design strategies for conservation. Our aim was to test the functionality of the barcoding approach in Orthalicidae. We used at least three specimens from seven Peruvian species (*Bostryx modestus*, *B. scalariformis*, *B. sordidus*, *B. conspersus*, *B. aguilari*, *B. turritus* and *Scutalus versicolor*). After total DNA extraction by CTAB method from foot muscle tissue, we amplified and sequenced a 706-bp fragment of the mitochondrial Cytochrome C oxidase subunit I (COI) gene (both strains) using COI universal primers (Folmer *et al.*, 1994). We surveyed the phylogenetic trees and evaluated genetic distance (K2p) among species. The topology of the tree showed a polyphyletic status of the three first species, with shared haplotypes, in contrast to the monophyletic status of the others. The intraspecific genetic distances within *B. conspersus*, *B. aguilari*, *B. turritus* and *Scutalus versicolor* were less than 2%, in contrast, the interspecific distances were strikingly high, making these COI sequences suitable barcodes for some Orthalicid species. However, it failed to define the limits in three species, (tagged by other DNA markers and morphology as the *Bostryx modestus* species complex) being the possible cause a recent episode

of diversification rather than a lack of sensitivity of this marker. Their diversification is thought to be related with Pleistocene changes and reinforced by ENSO activity occurred in the Peruvian coast. DNA barcoding was an efficient tool in this group, which had not received intensive taxonomic analyses.

MOLECULES VERSUS MORPHOLOGIES – A CONTEMPORARY FLORISTIC SURVEY OF CANADIAN SEAWEEDS

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This presentation will highlight successes and challenges to date in our DNA barcoding effort to generate a complete floristic account of the marine macroalgae in Canada. With ca. 5000 barcodes completed at the time of writing this abstract, we have uncovered more than 100 overlooked (cryptic) species/records in the Canadian flora including six unique to the Churchill region in the low Arctic. In addition to altering radically our perspectives on the actual numbers of species in Canadian waters, we are uncovering a bewildering array of tales including: putatively invasive species; a new family of red algae discovered in one of the most studied areas of Canada; new perspectives on algal distribution at both ecological and geographical scales; challenges to widely accepted morphological species concepts; rewriting phylogeographical hypotheses; resolving the dynamic nature of speciation and various hybrid populations; and uncovering key examples of niche exclusion with a corresponding restriction in phenotypic plasticity. I will present an overview of some of the highlights of our work with the aim of sharing the power of molecular-assisted alpha taxonomy in resolving species-level conundrums for a group of organisms that are notoriously impossible to identify using conventional approaches. I will also touch on some of the outstanding issues including: the difficulty in matching cryptic species to existing names (DNA from type collections); and the lack of success for COI as a barcode for green algae with a recommendation for an alternative marker. The latter has wider ramifications for barcoding across protistan taxa, which will also be discussed.

DNA BARCODING OF FUNGAL SPECIES FROM LEAF OF POPLARS

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Widespread use of hybrid poplar in north-central and north-eastern North America is limited by occurrence of diseases caused by different species of fungi. Among them, the haploid Coleomycete fungus *Septoria musiva* (telomorph *Mycosphaerella populorum*) is responsible for

leaf spot and canker disease, and the Uredinale *Melampsora medusae* causes the poplar leaf rust. However, stem cankers have recently been reported in new bioclimatic domains including the Fraser Valley in Bristish Columbia (B.C.), a previously *S. musiva*-free area. The occurrence of the hybrid *M. x columbiana* (*M. occidentalis x M. medusae*) and *M. medusae* is also suspected in B.C. By harvesting and mapping infected poplars and conducting DNA barcoding to assess *Septoria* and *Melampsora* species distribution, we can evaluate the risk of damage of these poplar pathogens and determine the risk of epidemics and the potential for eradicating these pathogens. The ITS rDNA barcode sequence used for fungal pathogens were generated from poplar leaves harvested in B.C. during the fall 2008. The results indicate that the native *Populus trichocarpa* is almost exclusively infected by the native *S. populicola* while the non-native *S. musiva* appears to be confined to hybrid poplars in plantations. Moreover this barcoding study revealed that the hybrid rust *M. x columbiana* is prevalent among native and hybrid poplars and that the native *M. occidentalis* seems to be rare. These results illustrate the potential for using fungal DNA barcodes ability to identify, evaluate and monitor the distribution and spread of these fungal pathogens and generate data that can be used for disease management decisions.

A TEMPLATE FOR FIELD DATA COLLECTION TO AID DNA BARCODING

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A significant proportion of samples for DNA barcoding is contributed by field-based operations doing regional biodiversity inventories and monitoring. While capable of supplying high quality specimens in large volumes, such sources rarely maintain collection databases and their effort spent on data parsing and conversion required to ensure compliance with the stringent barcode data standards may become prohibitive. As a measure to aid field researchers in aggregating and submitting collection information, a field data management spreadsheet has been developed to address the essential curatorial needs involved in field specimen processing. Its primary function is to facilitate spatiotemporal data tracking for the survey effort deployed, curation of collected lots and individual specimens, assembly of sampling and imaging arrays, and BOLD data submission.

The spreadsheet is built on the MS Excel platform and requires no specialized database training or software resources from its prospective users. Built-in formulas and macros offer versatility in data management. The establishment of hierarchical relationships between lot and specimen records and corresponding collecting events minimizes the need for repetitive data entry. A set of conversion tools allows outputting data in a variety of flat file formats compatible with BOLD and Darwin Core v.2 metadata standards. Several ancillary tools are offered, such as automatic generation of specimen and lot label printouts. Taxonomic nomenclature is validated against an imbedded reference checklist and geospatial data can be directly plotted in Google Earth. Although metadata requirements are regimented, there is sufficient flexibility to accommodate for exceptional data entry needs, such as digitization of provenance information from collection labels of museum specimens. Full functionality can be attained with a standalone computer workstation connected to laser printer (with archival paper).

While not intended to become a replacement for specialized collection databases, the spreadsheet has proven to save valuable field time and improve the quality of data capture. Its broader usage will not only enhance the efficiency of front-end stages of DNA barcoding, but will help address many logistical challenges of pre-museum collection management.

BIO-PEDAGOGY AND DNA BARCODING: THE CANADIAN NATIONAL MARKET SURVEY

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The rapidly accelerating pace of technological development is driving new discoveries in all areas of science, particularly in the life sciences. This presents a challenge for educators to maintain currency in their science-based curricula, especially as related to genomics, a field that relies heavily on bioinformatics and biotechnology. A further challenge lies in fostering student interest in these complex and varied domains of knowledge. Here, we describe a learner-centered approach to bioscience education that links teaching and research using DNA barcoding, a powerful new genomic tool for species identification and discovery. The pedagogical value of barcoding is presented as an example of applied evolution, one that is easily communicated to a diverse audience. Because of its simplicity and broad relevance, barcoding represents an ideal introduction to numerous fields of enquiry (e.g. biodiversity science, cell and molecular biology, forensics, taxonomy and systematics). Through collaborations with researchers, educators, their students (both secondary and post-secondary) and the media, we focused on the use of barcoding to detect market substitution in seafood. Broad coverage was achieved through the coordination of twelve specimen collection groups that collectively provided over 500 market samples for analysis. The project achieved a high level of student engagement, both through direct participation in the sample collection process and via E-Learning exercises that included the analysis of actual experimental data using the Barcode of Life Data Systems (BOLD, www.barcodinglife.org) and GenBank. Media engagement provided an open-learning aspect to the project that enhanced its social relevance. Consistent with earlier findings, this study provided evidence of market deception concerning seafood sold in Canada, but at a national scale. We conclude the market survey provided an effective student training opportunity because it addressed a question of social relevance and as a result stimulated an interest in citizen science. Various considerations for undertaking such a survey are discussed.

PROTOCOLS FOR DRY DNA STORAGE AND SHIPMENT AT ROOM TEMPERATURE

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The globalization of DNA barcoding will require core analytical facilities to develop cost-efficient, effective protocols for the shipment and archival storage of DNA extracts and PCR products. We evaluated two dry-state DNA stabilization systems, commercial Biomatrica® plates and home-made trehalose plates, on 96-well panels of insect DNA stored at 56°C and at room temperature. Controls included unprotected samples that were stored dry at room temperature and at 56°C,

and diluted samples held at 4°C and at   -20°C. PCR and selective sequencing were performed at 8 points over a 1-year interval to test the condition of DNA extracts. Biomatrica® provided better protection of DNA at 56°C and at room temperature than trehalose, especially for diluted samples. However, trehalose provides a very cost-effective option with only a minor loss in DNA stability. All dried samples showed some DNA degradation as a result of the series of rehydration events over the year, in comparison to DNA stored at -20°C without freeze-thaw events. Although it is premature to advocate a transition to DNA storage at room temperature, dry storage provides an additional layer of security for frozen samples, protecting them from degradation in the event of freezer failure.

BEYOND BARCODING – SECURE DNA STORAGE

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In DNA barcoding projects enormous amounts of DNA samples are being processed worldwide. Appropriate storage of any material remaining after sequences have been gained is essential to allow subsequent verification and addition of data. While barcoding technologies and data analysis methods improve continually, the influence of different storage conditions on the quality of DNA samples is still insufficiently investigated.

The German Science Foundation (DFG) supports a DNA Bank Network (www.dnabank-network.org) as a service facility for research in the life sciences. To optimize the storage conditions in our DNA banks we investigated DNA degradation in sample storage at five temperatures (room temperature, +4°C, -20°C, -80°C, and –196°C in liquid nitrogen), and in three different agents (buffer, water and QIAsafe DNA Tubes). The influence of sample dehydration, protective additives (trehalose) as well as of repeated freezing and thawing was also examined. DNA degradation was measured by a quantitative real-time polymerase chain reaction (qPCR) method, which has the ability to estimate accurately the amounts of targeted fragments in a sample. In our experiments four fragments of the mitochondrial genome (including the barcoding region) in the range of 350 to 2350 base pairs were used as indicators for DNA degradation. Here we present the results of our experiments after one year of storage.

THE INFORMATION SYSTEM OF DNA BARCODE OF LIFE IN CHINA

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The aim of DNA barcode of life is to utilize a relatively short DNA segment to identify species around the world. To accomplish this goal, extensive efforts are needed on field expedition, methodology, experiments as well as information system. In China, besides sample collection, method standardization, and species identification, we have also planned and started to construct an comprehensive information system for the data collection, data curation, data exchange, information integration, and bioinformatics, etc. As for the data collection, we have built up a PHP-MySQL based platform to accommodate the raw data submitted from the contributors around China, either through single entry submission or batch submission. The data fields include those recommended by CBOL. To meet Chinese users' need, we used both English and Chinese tags. The data curation system is to make the submitted data standardization, and then transfer to the standard China BOL database. The database schema of the curated data is constructing according to the schema of BOLD. Data exchange is to obtain the global data into China site and send data of China site to the world. Currently, we have received the data from BOLD via XML file. The data has been imported into our database, and can be accessed now. Meanwhile, our data curation in on processing and the curated data will soon be transferred to BOLD. The BOL information system in China has also included an information integration platform and a bioinformatics platform. Information integration is to collect as many as useful information and links of a given species for the user, via the current web technology. Currently, the related nucleotide and protein information, literatures, and some other useful links are provided. Bioinformatics are mainly focus on database search, sequence comparison and phylogenetics. We have setup a platform containing BLAST, ClustalW and program for Neighbor-Joining tree construction. In total, the information system of DNA barcode of life in China is aim to utilize the data collection, data curation, data analysis and data exchange to the world.

QUALITY ASSURANCE AND CROWD CONTROL IN A HIGH-THROUGHPUT DNA BARCODING FACILITY

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Since 2006, the Canadian Center for DNA Barcoding (CCDB) has gathered records from about 125K specimens a year. In order to support the varied iBOL research programs, the CCDB must increase its production to 500K specimens per year by 2013. New policies and procedures are being adopted which will meet this challenge while increasing data quality without inflating the cost of analysis. Several key analytical steps have been modified with the adoption of products that provide robust results under a wider range of conditions. Reorganization of workflows in late March 2009 has already led to a two-fold rise in production as measured by the number of

barcode sequences uploaded to BOLD that equals nearly 21K barcodes per technician per year. This increase has been achieved without major changes in staff and equipment resources through targeted resource allocation and regimented work schedules that eliminated competition for equipment and improved staff efficiency. A new approach towards the management of trace files distribution in conjunction with revised sequence editing and assembly protocols, have also contributed to the production increase.

Technical Session Abstracts – Wednesday

Barcoding the Trees of Africa

BARCODING THREATENED PLANT SPECIES OF WEST AFRICA – NIGERIA AS A CASE STUDY

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The African continent is endowed with rich biodiversity. The status of these resources is constantly being altered in the ecosystems as they gradually decline from abundance to threatened, endangered and near extinct. The biological diversity of each country is a valuable and vulnerable natural resource, and knowledge about biodiversity including the ability to identify organisms are global public goods. Numerous potentials abound in Africa for DNA barcoding research for sustainable development aimed at meeting the Millennium Development Goals.

Many countries of Africa have established protected areas inter alia, as an effective measure to conserve natural biodiversity. Today more than 8.6% of the continents land area is protected to stem the tide of biodiversity loss in West/Central Africa (IUCN-WCRA 2009). This approach encourages *in situ* and *ex situ* conservation of biological species generally, especially the endangered, threatened or near extinct, thus, making vital contributions to the conservation of the worlds' natural resources.

Nigeria is a country with an internationally recognized abundance of rich biodiversity, with bio-diverse natural ecosystems that range from natural lowland and montane forests to important freshwater wetlands, to savannas and high-altitude plateaus, to mangroves and coastal areas. Protected areas in Nigeria significantly represent the country's biodiversity, hence the need to further protect and conserve these resources through DNA Barcoding

The aim of this work is to catalogue, barcode and produce reference barcodes of all the threatened plant species present in all protected areas of West Africa. The National Centre for Genetic Resources and Biotechnology (NACGRAB), among other collaborating institutions in the West and Central African region are strengthening their taxonomic infrastructure in order to embark on this project. A preliminary survey of threatened species in all protected areas in Nigeria will be conducted using the IUCN's red list as a guide. Subsequent DNA Barcoding of threatened species will enhance knowledge on the biological data and diversity. Conservation potentials will be unveiled, and DNA barcode sequences made available to public data bases such as BOLD system.

This collaborative work is expected to promote and strengthen supportive networks, enhance Access and Benefit Sharing (ABS) opportunities and provide information on the status of threatened plant species in the region as stipulated by the CBD objectives
Key words: DNA Barcoding, biodiversity, protected areas, threatened plant species.

EXPLAINING TREE AND SHRUB REGIONAL DIVERSITY PATTERNS IN THE KRUGER NATIONAL PARK (SOUTH AFRICA)

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A large-scale effort has been undertaken during 2006 to 2008 to barcode all the trees and shrubs of the Kruger National Park (KNP), South Africa. This project has generated ca. 630 barcodes and a complete phylogenetic tree using both rbcL and matK. The KNP is situated within the savanna biome and is one of the largest national parks in the world. The southern part of the park is also part of the Maputaland-Pondoland-Albany Hotspot, an important center of plant endemism. Apart from Kruger's obvious botanical interest, it constitutes an excellent area for research because a lot of spatially explicit environmental data have been collected and are available from the park's Scientific Services. Considering the above, our aim is to investigate and understand the regional patterns of tree and shrub diversity in the KNP, taking into account not only the environmental factors possibly giving rise to these patterns, but also the potential role of evolution through the calculation of the phylogenetic diversity. We address the following main questions: (1) Do specific tree and shrub diversity patterns exist in the KNP? (2) What are the main environmental correlates with these diversity patterns? An overview of the management of samples and the electronic DNA barcoding database of the flora of the KNP will also be discussed.

STRENGTHENING TREEBOL AFRICA INITIATIVE

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The TreeBOL Africa is part of the global initiative with a major goal of establishing a network of African scientists and institutions working in the field of DNA barcoding. TreeBOL- the African Campaign was held in the Department of Botany and Plant Biotechnology, University of Johannesburg in October 2008. At the end of the meeting there was general consensus among participants to establish four regional working groups namely; southern Africa, west and central Africa, east Africa and the Indian Ocean including Madagascar and Mauritius that would lead the primary data collection efforts. A list of high-priority barcoding projects was developed that included but not limited to traded species (CITES, timber, and other protected species like cycads), medicinal plants, invasive species (weeds), rare/endangered species and biodiversity hotspots. One of the limiting factors is the shortage of capacities and facilities. All regions of Africa are not in equal standing regarding the level of research in molecular genetics and related fields. DNA

barcoding requires state of the art laboratory and there is also a need to have DNA banks. The critical manpower need to accomplish the objective of the initiative also calls for capacity building at all levels. The first attempt towards capacity building was already taken up by the University of Johannesburg in January 2009; DNA barcoding and grant writing skill course was conducted for participants from the different regions of Africa. In conclusion, we want to greatly emphasize that there is a lot needed to bring all concerned and interested stakeholders from within Africa and the rest of the world to strengthen the TreeBOL Africa initiative.

TREE BOL LAGOS: BARCODING OF TREES IN LAGOS

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Nigeria's biodiversity is one of the most endowed in Africa but also one of the most threatened by human activities and climatic change. Today, many tree species face extinction or severe genetic loss and for most of the endangered species, little effort is being made for their conservation. DNA barcoding is the use of a short gene sequence from a standardized position in the genome to identify species. It is a useful tool for taxonomic research and an innovative approach to many practical problems that require species identification.

The aim of this work is to carry out exploration of the tree species in Lagos and barcode all the available varieties with a view to sharing its DNA barcode sequence and data in a public data base. The objectives however, are: to explore the diversity of tree species, to process the tissues so as to obtain DNA barcode sequences and to share the DNA barcode sequence and data about its voucher specimen in the web database.

In order to achieve this, fresh plant samples were collected from the University of Lagos and its environs, identified, preserved and voucher specimens were deposited at the University of Lagos herbarium. Some of the samples were also preserved in silica gel for DNA extraction purpose. The extracted DNA samples were purified; size separated by gel electrophoresis and quantified using spectrophotometer. Polymerase Chain Reaction (PCR) was then carried out on the samples and the products were subjected to DNA sequencing so as to generate DNA barcodes following standard laboratory protocols.

At the end of this experiment, different sizes of DNA samples were obtained and photographed using a UV documentation machine and a Polaroid film. Also, DNA sequences were obtained from which barcodes are then generated to enhance rapid identification of trees in Lagos and sequences are being deposited at the gene bank and would also be deposited at TREE BOL for easy access by researchers and students.

STRENGTHENING AFRICA'S CAPACITY IN DNA TECHNOLOGIES FOR BIODIVERSITY RESEARCH AND SUSTAINABLE USE

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Africa's biodiversity is one of the most extraordinary in the world, but also one of the most threatened by human activities (population growth, over-exploitation, logging) and global change (desertification, climatic warming). For these reasons, Africa hosts eight global biodiversity hotspots, that is, the richest and most endangered reservoirs of plant and animal life on earth. While biodiversity is disappearing alarmingly fast, there have also been fantastic technological developments that can help reverse biodiversity loss – but the problem is that these novel technologies are not easily accessible by countries rich in biodiversity but constrained in resources. Our project will strengthen research frameworks for international, regional and inter-institutional co-operation in Africa in the in field of DNA barcoding technology for biodiversity science. To make efficient use of these types of DNA technologies in research and biodiversity conservation, Africa needs to increase significantly its expertise in this scientific area, and policies should be put in place to assist these developments. This is the challenge that our project will address. Our project will focus on networking, which will be facilitated by the creation of a virtual Laboratory for African Biodiversity (LAB) distributed among the partners. This networking tool will be important for developing the use of genetics/genomics – and DNA barcoding in particular – to aid biodiversity science for sustainable development. This 'LAB' will facilitate the exchange of material, expertise, students and personnel. It will build on an existing network comprising scientific institutions actively involved with the aim of DNA barcoding all tree species, TreeBOL (Tree Barcoding of Life). Our project will focus on DNA Barcoding African Trees (BAT). The main target groups of this project are educational organizations (universities), research organizations/researchers and students from Africa.

THE NATIVE BEES OF MÉXICO AND THE DNA BARCODE OF LIFE PROJECT

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Bees are considered some of the more important pollinators of wild and cultivate plants; these provide essential ecosystem service that results in the out-crossing and sexual reproduction of many plants. The rich bee fauna of Mexico encompasses 6 families and 153 genera, with approximately 1839 currently recognized species. The distribution of the richness between families is as follows: Apidae 606 species, Andrenidae 535, Megachilidae 355, Halictidae 229, Colletidae 103 and Melittidae 11. The 10 largest genera in Mexico are Perdita 233 species, Megachile 112, Protandrena 104, Andrena 94, Centris 54, Colletes 51, Melissodes 48, Callopsis 45, Exomalopsis 42, Coelioxys 36 and Xylocopa 36.

Due to gaps in collecting and the paucity of current revisions, the apifauna of Mexico is certainly much richer, perhaps well in excess of 2000 species. The diversity of bees in Mexico appears intermediate between that of the United States and countries to the south. The rich bee fauna of Mexico is in part the result of its unique position at the juncture of the Nearctic and Neotropical regions, with strong contributions of both temperate and tropical elements plus a unique Mesoamerican component.

There are four major collections in México that have holdings of bees. These are Instituto de Biología, UNAM (Chamela), Facultad de Ciencias UNAM; ECOSUR and the Universidad Autónoma de Yucatán. As a general estimation, only 20% to 30% of the species of most groups are represented by specimens less than 20 years old.

Although their ecological, economic importance, bees are facing threats from growing loss of habitats principally in the tropical areas. Thus, it is urgent to conduct more taxonomic studies considering the DNA Barcode of Life project, that could help taxonomic studies providing practical method for the identification of species. We are participating in the BeeBOL initiative, with this we hoped to obtain the DNA molecular sequences of bee species of Mexico. At the moment, there are around 200 bee species in The Barcode of Life Data Systems (BOLD). The following stage is the accomplishment of new faunísticos projects, to reunite recent specimens of Mexican bees to be sequence, having the strategy of include species of economic or biological importance, as it is the case of the species that are important pollinators for cultivating plants.

APPLYING DNA BARCODING AND MORPHOLOGY TOWARD IMPROVING THE TAXONOMY OF THE CLEPTOPARASITIC BEE GENUS NOMADA

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Nomada (Hymenoptera: Apidae) is the largest genus of cleptoparasitic bees, with a world-wide (but primarily Holarctic) distribution. These bees commonly parasitize the largest genus of provisioning bees, *Andrena*, with a minority of species parasitic instead on at least three other bee families. *Nomada* contains approximately 800 presently described species, with many more awaiting formal description. Species identification in this group often is difficult due to the lack of reliable taxonomic tools. In order to improve the taxonomy of this genus, we gathered and analyzed DNA barcode data from the standard animal COI locus.

Our efforts focused primarily on the ruficornis species-group, abundant in the Nearctic and fraught with taxonomic issues including species delimitation and gender association. We obtained sequence data from more than 1,200 individuals sampled over a wide geographical range across much of the United States and Canada. We interpreted our results in light of specific morphological characters and variation present in these specimens. We discuss examples where our molecular data help establish species differences and nomenclatural synonymies, as well as cases in which these data may have more difficulty with species delimitation and identification.

ESTIMATING DIVERSTIY: DNA BARCODING AND MORPHOSPECIES

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At a time when biodiversity loss and accompanying pollinator declines are becoming increasingly widespread, the need for accurate and rapid identifications of taxa is paramount. Bees are an integral part of ecosystems, and contribute billions of dollars per year to the agricultural sector, yet our knowledge of the majority of 19 500 bee species remains inadequate. Here I review the capabilities of parataxonomy and the efficiency of DNA barcoding in quickly and accurately identifying species, specifically the Apoidea of Thailand. This group presents an interesting challenge as almost no regional literature exists. To date, the most comprehensive regional species checklist suggests 37 genera and 130 species inhabit the country. Our preliminary data, taken from 15 of the 24 National Parks, most of them concentrated in the Central and Northern regions, has revealed a total of 121 distinct barcode species. At least 8 genera now have more species known through barcoding than from traditional taxonomic work and a new species of *Systropha* has been discovered, though most genera remain to have species level idenfications performed. In addition a morphological species sort was performed for three of the genera

present. Comparison of datasets showed an accuracy rate of 57% (one morphospecies to one barcode species). Barcode data was able to associate sexes and castes where a morphospecies sort failed to account for dimorphism and allometric differences. The benefits of DNA barcoding are reviewed, and possible extensions of this study are discussed.

BARCODING BEES WITH EMPHASIS ON CANADIAN MEGACHILE: IMPLICATIONS, RESOLUTIONS AND NEW ASSOCIATIONS

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Bees are considered one of the most important groups of organisms due to their keystone role as pollinators in most terrestrial ecosystems. Despite this, much remains to be resolved in bee systematics, even in the relatively well known fauna of Canada (approximately 800-900 species). DNA barcoding has much to offer towards these efforts, as demonstrated in a recent revision of the Canadian species of Megachile. This genus contains the well known alfalfa leafcutter bee, an economically important species that is used to pollinate a variety of crops including alfalfa and lowbush blueberry. DNA barcoding was used to great effect to: 1) discover and/or verify the unknown sex of three species, 2) associate the male and female of another, resulting in a synonymy, and 3) raise a previously recognized "subspecies" to species level. As a result, the taxonomy of the Canadian species is now fully resolved, permitting recognition of natural range expansions and the arrival of invasive species into the country. Implications of barcoding on other bee genera will be discussed.

DNA BARCODING A NIGHTMARE TAXON: A CASE STUDY FROM BEES

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Bees worldwide play a fundamental role in terrestrial ecosystems as pollinators of wildflowers and agricultural crops. The cosmopolitan family Halictidae, also known as sweat bees, is by far the most commonly collected, morphologically monotonous, and behaviourally diverse family of bees. Over 4000 species of sweat bee are known worldwide. They occur on all continents, except Antarctica, and range from the tropics to the arctic. In both temperate and tropical habitats, halictids can constitute 40–85 % of individual bees collected in faunal studies. For many species of plants they are the most important pollinators. Multiple origins and losses of eusociality among sweat bees with several possible intermediate social systems makes them the best models for studying the factors that lead to eusociality.

Unfortunately, halictids are notoriously difficult to identify to species. Taxonomic difficulties in sweat bees are especially severe for the monolithic genus *Lasioglossum* (> 1700 sp.). DNA

barcodes are helping to rapidly resolve long-standing taxonomic problems in these bees. The most difficult group of bees in North America, *Lasioglossum* subgenus *Dialictus*, is presented as a case study for incorporating DNA barcodes into a taxonomic framework. DNA barcodes are a valuable tool for testing morphological assessments of species identity and associating sexes. DNA barcoding has lead to 49 new synonymies in a revision of Canadian *Dialictus*. Six new and two resurrected species of Dialictus have already been described using DNA barcodes and 16 additional new species will result from the Canadian revision. Preliminary DNA barcoding of Mexican *Dialictus* already reveals more species than have been previously recorded for the country.

MOLECULAR CHARACTERIZATION OF HONEYBEES, APIS MELLIFERA RACES FROM KENYA USING BARCODING MARKERS

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The three races of the honeybee Apis mellifera Linnaeus in Kenya (A.m. scutellata, A.m. monticola and A.m. litorea) differ from each other with respect to size, cubital index and abdominal colour banding pattern. Based on samples collected from different geographical and ecological zones throughout Kenya ranging from lowlands of the coastal regions to the highland regions of central Kenya, these differences were used to assess the extent on interbreeding and hybridization between the races using mitochondrial COI gene variations. The alignment of the sequences was performed by Clustal W program (Jeanmougin et al., 1998). Results show minimal variations in COI gene region important for species level identification of Apis mellifera. Sequence data analysis indicated there was hybridization among the three races of A. mellifera in Kenya due to swarming and migration under seasonal pressure.

MORPHOMETRICS AND DNA BARCODING OF STINGLESS BEES (APIDAE: MELIPONINAE) IN THREE SELECTED FORESTS IN KENYA

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Stingless honeybee keeping is an income generating enterprise with an indirect potential of achieving the goal of forest and biodiversity conservation in Kenya. However, little information is available on species diversity and spatial distribution of stingless bees. This study describes analysis of samples collected from Arabuko sokoke, Mwingi and Kakamega forests, ranging from low, middle to high altitude, respectively using morphometrics and molecular approaches. Fourteen morphometric characters were measured to determine the extent of morphological

variation of stingless bees in Kenya. The morphometric variables differentiated three populations of *Hypotrigona gribodoi* and *Meliponula bocandei* from three geographically distinct regions in Kenya.

H. gribodoi samples from three localities were separated using morphometric data by applying PCA (Principal Component Analysis) and CVA (Canonical Variate Analyis) into two population groups, Kakamega population was distinct whereas, Mwingi & Coast separated partially. Three populations of *M. bocandei* were separated into two groups on the PCA but separated further into three distinct groups on CVA. In addition, mitochondrial DNA sequence data for Cytochrome c Oxidase I (COI) generated in accordance with standards applied by the Barcode of Life Data systems (BOLD). Sequences from the same species clustered together when genetic distance-based cluster analysis was applied. Intraspecific divergence was less than 2% compared to high interspecific divergence of greater than 8.6%. The distance between groups analysis shows that *H. gribodoi* is closely related to *D. schimidti* with a distance of 0.086 compared to *M. bocandei* with 0.119.

THE CAMPAIGN TO BARCODE THE BEES OF THE WORLD: PROGRESS, PROBLEMS, PROGNOSIS

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Bees are the most important pollinators in the world and, with approaching 20,000 described species, their identification is not a trivial activity. In particular, there are many genera with hundreds of species and several with over 1000. Placing species names on bees in these genera is often extremely difficult, if not impossible. Consequently, DNA barcoding promises to be a great boon to researchers and agriculturalists wishing to put names on bees.

In May 2008 a campaign to barcode the bees of the world was launched at a CBOL-funded meeting held at York University, Toronto, Canada.   A dozen bee, barcoding and agricultural experts met and another three bee taxonomists sent in prepared presentations. A steering committee was formed, a website constructed and a set of operating principles agreed upon.

We now have at least one barcode sequence from 60% of the world's 480 bee genera and over 10% of the world's species.   Over 2000 distinct clusters have been found for approximately 1550 nominal species: barcoding is discovering a large number of previously unidentified species. All parts of the globe are represented in the dataset, other than Antarctica where there are no bees. In short, considerable progress has been made in a short time.

Problems associated with the campaign are mostly those associated with rapid growth of a database with insufficient personnel available for identification: many errors remain that require correcting. These issues will be solved shortly with the hiring of a full time database manager as of September.   Other problems have readily been solved: the recognition of heavy Wolbachia infestations in bees is easy, and leads to other interesting questions.

The prognosis for a bee barcode database that is as complete as is reasonable is good: we have a team of researchers throughout the world ready and able to obtain material and to perform the required species-level identifications.

BARCODING REVEALS CRYPTIC BUMBLE BEE SPECIES DIVERSITY

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Members of the subgenus *Bombus sensu stricto* (including *Bombus lucorum sensu lato* plus *Bombus terrestris*) are amongst the most abundant and widespread European bumble bees. However, their species diversity is controversial due to the extreme difficulty or inability morphologically to identify the majority of individuals to species. Definitive field identification is virtually impossible. We undertook a character-based phylogenetic analyses of 700 bp of mitochondrial cytochrome oxidase I DNA sequences (CO1) from 37 individuals of *Bombus s.str.* spread across their sympatric European range, that provides unequivocal support for 4 taxa (*B. cryptarum*, *B. lucorum*, *B. magnus* and *B. terrestris*) with 3-22 diagnostic DNA base pair sites per species. Inclusion of 20 Irish specimens to the dataset revealed greater than 2.3% sequence divergence between taxa and less than 1.3% within taxa, suggesting that distance-based DNA barcoding could be used to identify species. We developed a rapid and cheap PCR-RFLP based method for unequivocally distinguishing amongst these four cryptic European taxa of this subgenus and used it to analyse 391 females of the former three species collected across Ireland, all of which could be unambiguously assigned to species. *Bombus lucorum* was the most widely distributed and abundant of the *cryptarum-lucorum-magnus* species complex, comprising 56% of individuals, though it was significantly less abundant at higher altitudes (>200 m) whilst *B. cryptarum* was relatively more abundant at higher altitudes. *Bombus magnus* was rarely encountered at urban sites. COI DNA sequences are clearly able to identify these cryptic bumble bee species across their European range (Murray *et al.* (2008) Conservation Genetics 9, 653-666), and are a testament to the value of DNA barcoding for species identification for this group of closely related bee species.

POLLINATORS AT RISK: IDENTIFYING CRYPTIC SPECIES IN NATIVE BEES FROM MEXICO (HYMENOPTERA:MELIPONINI)

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The stingless bees (Meliponini) are a key group of social pollinators in tropical ecosystems. Unlike honey bees, colonies of stingless bees do not migrate or abscond and some species are highly dependent on intact rainforests for nesting and food resources. Thus, deforestation and habitat deterioration are threatening the survival of many stingless bee species. In Mexico, several species are used in stingless beekeeping that is an important social and economic activity for many indigenous groups. Fourty six species of stingless bees have been reported for Mexico but significant phenotypic variation exists within many species. Although population genetic studies of Mexican stingless bees are scarce, the evidence suggests that cryptic species may be found in some genera because their taxonomic status is difficult to assess based on morphology alone.

The aim of our new project is to provide a revised list of the species of stingless bees of Mexico, their geographic distributions and recommend schemes for conservation and management of the endangered ones. We propose analysing three molecular markers: *Cox1*, *ITS-1* and *EF-1a* in three widely distributed genera where cryptic species may be found (*Plebeia*, *Nannotrigona*, *Scaptotrigona*). The information from the barcoding markers will be compared and combined with that obtained from microsatellite analyses and morphology to define taxonomic units. Results from this important taxon will also be incorporated into the Bee-Bol initiative.

NICHE PARTITIONING BASED ON NESTING BIOLOGY IN TWIG-NESTING CARPENTERS BEE REVEALED BY CONGRUENT VARIATION IN BEHAVIOUR, MORPHOLOGY, AND DNA BARCODES

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Ceratina dupla and C. calcarata are two of the most common bees in eastern North America. They are sympatric across their range and remarkably similar in morphology, nesting behaviour, and flower preferences. Although doubt has been expressed as to whether these are separate species, variation in cytochrome oxidase (COI) sequences confirms both their specific status and a key taxonomic character used to distinguish them (Rehan and Richards 2008). In addition, greater COI sequence variability in C. dupla suggests the possibility that this species may comprise multiple genetic lineages, possibly multiple species. To understand how at least two bee species with

almost indistinguishable habits can exist in sympatry, we initiated field studies comparing their nesting biology, in conjunction with DNA barcoding of specimens studied in the field. We found clear evidence for niche separation based on differences in nest site selection at field sites occupied by both species. C. calcarata tends to nest in raspberry canes in shady sites, has smaller clutch sizes, and brood are more likely to be parasitized, whereas C. dupla is more often found nesting in warm sunny sites in teasel stems, has higher clutch sizes, and lower rates of brood parasitism. Field studies also suggested greater variability in the nesting habits of C. dupla, with a few small females nesting very early and very late in the season. DNA barcoding then revealed the existence of two separate lineages, each with distinct phenology and morphometry, which we refer to as dupla1 and dupla2. Dupla1 is rarer, smaller, begins nesting considerably earlier, and is probably bivoltine, whereas dupla2 is common, larger, nests later, and is univoltine. Thus, the combination of field and molecular studies reveals three sympatric species of morphologically similar twig-nesting carpenter bees, which apparently reduce inter-specific competition by niche partitioning based on nest site selection and phenology.

THE BEE FAUNA OF GUATEMALA AND THE IMPORTANCE OF PARTICIPATING IN THE BARCODE OF LIFE

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The known bee fauna of Guatemala, is represented by 325 species. However, we consider the number could exceed 500 species after more faunistic, revisionary and molecular studies are conducted. These bee species belong to 101 genera and 5 families. The distribution of the richness between families is: Apidae 193 species, Halictidae 49, Megachilidae 57, Colletidae 18 and Andrenidae 8. The 15 most speciose genera comprise 50.7% of the fauna in Guatemala. Among those, the five most diverse are Megachile with 25 species, Centris 16, Lasioglossum 16, Xylocopa 15 and Euglossa 13. The stingless bees are an important group with 38 species.

Bees are considered the most important pollinators of cultivated and wild plants. Also, in several cultures, including some in Guatemala, the products from the stingless bee hives are used as a source of medicine and food. Although their importance, bees are threatened by the increasing loss of habitats in Guatemala. Thus, it is urgent to promote and implement actions directed to their conservation. The lack of taxonomic studies makes the application of effective conservation strategies difficult. It is therefore necessary to conduct more taxonomic studies that help in the identification of bee species.

Since 2003, our group has conducted some faunistic studies in the country, focusing the collecting efforts on three sub-tribes of the Apidae family. Most of the specimens have been identified based on morphological data. Now, we are conducting a research to evaluate the morphological variability observed in the Melipona species of Guatemala through morphometric and molecular analysis. We consider that the participation in the DNA Barcode of Life project will increase our

capacity for the identification of bees. Up to now, we have already sent specimens of stingless bees to collaborate with the Bee Barcode of Life Initiative.

FISH-BOL

US FDA VALIDATION OF DNA BARCODING TO PROMOTE SEAFOOD SAFETY AND COMBAT ECONOMIC ADULTERATION

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The United States Food and Drug Administration is responsible for assuring that the nation's food supply is both safe and accurately labeled. This task is particularly challenging in the case of seafood where a large variety of species are marketed, a high percentage of this commodity is imported, and marketed product is often processed to a point where traditional morphologic species determination is not possible.

New methods that allow accurate and rapid species identifications are critical for both food borne illness investigations and for the prevention of deceptive practices such as those where species are intentionally mislabeled to circumvent import restrictions or for resale as species of higher value. But, any new methods to be used for regulatory compliance must be both standardized and adequately validated.

DNA Barcoding using the Folmer fragment of the cytochrome c oxidase subunit 1 (COI) mitochondrial gene was an ideal method of choice to evaluate for these applications because of the large number of sequences and publications already available from international consortia and campaigns such as CBOL and FISH-BOL. In collaboration with the Canadian Center for DNA Barcoding and the Smithsonian Institution's Laboratory of Analytical Biology, the FDA is in the process of validating a standardized method for DNA Barcode generation for regulatory use. In addition, in partnership with the US National Marine Fisheries Service and the Smithsonian National Museum of Natural History, the FDA is building a vouchered library of regulatory seafood species standards, which will include COI sequences, that will be added to its on-line public resource, the Regulatory Fish Encyclopedia.

THE CEAMARC SURVEY: BARCODES AS A MULTI LEVEL TOOL

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The CAML-CEAMARC cruises allowed to prospect depths yet unexplored in the Eastern part of the Antarctic continental shelf, off Terre Adelie in the winter 2007-2008. Seven hundred teleost specimens (including very rare species) collected during these cruises were sequenced for the mitochondrial cytochrome oxydase I gene (COI). The sampling was completed with carefully chosen specimens from previous campaigns to allow a better exploration of intra and interspecific variability. Moreover, as using a single marker cannot bring definitive answers to taxonomic problems, several other markers (cytochrome b, D-loop, the nuclear rhodopsin retrogene, and a new nuclear marker, pkd1) were sequenced for for several carefully chosen groups to test the congruence among the results for different genes, and explore the efficiency of the COI gene at recovering species limits.

The partial COI gene yields reliable identification in most Antarctic teleost families, although several groups of species cannot be differentiated through its use alone. In particular, its variability is very low in artedidraconids, although there are a few molecular synapomorphies for most of the species investigated. In genus Trematomus, almost all species are well separated except for two pairs of more closely related species. For zoarcids and liparids, the results of barcoding are in agreement with in-depth morphological study, and will be an invaluable asset for the identification of these hard to identify species once a reasonably complete reference dataset is available. A careful confrontation between morphological and molecular data for our specimens allowed to add numerous well identified specimens and sequences in the BOL database for under-represented groups in the region; it helped to pinpoint the specimens that needed to be re-checked morphologically, and highlighted groups where systematic sampling for barcoding is necessary for the good identification of the specimens.

BARCODING ARGENTINE FISHES

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As a contribution to FISH-BOL we analyzed 564 specimens of fishes collected in Argentine waters, representing 125 species. A standard 652 bp BARCODE fragment of the 5' mitochondrial cytochrome c oxidase subunit I gene was amplified and bi-directionally sequenced. All sequence assemblies, electropherogram (trace) files, primer sequences and specimen provenance data were deposited in the Barcode of Life Database (BOLD). This included digital images of morphological voucher specimens as well as GPS coordinates for all specimen collection localities. Using the suite of analytical tools available on BOLD, a Kimura two-parameter (K2P) genetic distance matrix was calculated to estimate the relative sequence divergences within and among species. A Neighbour-joining (NJ) phenogram was also constructed from this distance matrix.

Nearly all species exhibited unique BARCODE haplotypes or monophyletic clusters of very closely related haplotypes, which permitted the discrimination of 98.4% of species. K2P genetic distances averaged just 0.21% within species, but averaged 3.92% within genera, 17.04% within families, 23.98% within orders and 25.23% within classes. Only the skates *Psammobatis rudis* and *P. normani* could not be separated from each other using barcodes. The use of barcodes within an integrative taxonomic framework confirmed the identification of a new species of longnose skate (*Dipturus argentinensis*) from the Argentine Sea and also permitted the recognition of the Brazilian cusk eel (*Genypterus brasiliensis*) as a valid species.

Although some groups are highlighted for further taxonomic analysis these results support the utility of DNA barcodes for regional species identification of fishes. When comparing these results to other projects on BOLD, standardizing the application of names across collections/regions emerges as a significant challenge for FISH-BOL. However, we conclude this long-standing issue is most efficiently addressed through DNA barcoding.

IDENTIFYING CORAL REEF FISH LARVAE THROUGH DNA BARCODING: A TEST CASE WITH THE FAMILIES ACANTHURIDAE AND HOLOCENTRIDAE

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The efficacy of the barcoding approach in identifying early stages of coral reef fishes is explored here. A reference collection for the Pacific Society Islands has been constituted based on exhaustive sampling of adults from two common reef fish families Acanthuridae and Holocentridae. A segment of 650 base pairs of the cytochrome c oxidase I gene (COI) has been sequenced for the 22 species of Acanthuridae (53 specimens) and 16 species of Holocentridae (53 specimens) of the region. Divergence between congeneric species was on average 20-fold to 87-fold higher than divergence between conspecific sequences and that each species was represented by a cluster of tightly related sequences. Once validated, this set of DNA-identifiers was used to identify 46 larvae of both families sampled by trawling in the pelagic zone around the Society Islands. With morphological diagnostic characters, only 11 larvae could be identified to the genus, while all larvae sequenced could be identified to species using DNA-barcodes. From those identifications it was apparent that pools of larvae collected in each sample constitute multi-specific assemblages. Furthermore, no additional species compared to adult reef communities were sampled in larval pools, suggesting that the larval assemblages originated from adult communities on neighboring reefs.

SEQUENCE DIVERGENCE AT CO-1 AND CYT-B mtDNA ON DIFFERENT TAXONOMIC LEVELS AND GENETICS OF SPECIATION AND PHYLOGENETICS

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Genetic divergence for Co-1 and Cyt-b are analyzed. Using a database of p-distances and similar measures, genetic divergence of populations (1) and taxa of different rank, e.g. subspecies, semispecies or/and sibling species (2), species within a genus (3), species from different genera within a family (4), and species from separate families within an order (5) have been compared.

Empirical data for 18,192 vertebrate and invertebrate animal species demonstrate that the data are realistic and interpretable when p-distance or its derivates are used. The focus was on vertebrates and fish species in particular, and the newest dataset obtained in the framework of CBOL. Various and increasing levels of genetic divergence of the sequences of the two genes Co-1 and Cyt-b in the five groups revealed. Mean unweighted scores of p-distances for five groups are: Co-1 (1) 0.72±0.16, (2) 3.78±1.18, (3) 10.87±0.66, (4) 15.00±0.90, (5) 19.97±0.80 and Cyt-b (1)

1.46±0.34, (2) 5.35±0.95, (3) 10.46±0.96, (4) 17.99±1.33 (5) 26.36±3.88. This testifies to the applicability of p-distance for most intraspecies and interspecies comparisons of genetic divergence up to the order level; in other words vast data reviewed provide theoretical and empirical background for DNA barcoding.

Ample evidence showing different and nonuniform evolution rates of these and other genes and their various regions have obtained. The results of the analysis of the nucleotide divergence within species and higher taxa are in a good agreement with previous results and suggest that in animals, phyletic evolution is likely to prevail at the molecular level, and speciation mainly corresponds to a geographic or divergence model, D1. The prevalence of the D1 speciation mode does not mean that other modes are absent. There are at least seven possible modes of speciation. Can we recognize them formally is a key question for a theory. An approach is suggested allowing establishing a genetic theory of speciation.

DNA BARCODING INDIAN FISHES

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The bounty of marine biodiversity, which is exploited from 2.02 million sq. km of the Exclusive Economic Zone (EEZ) of India, constitutes one of the largest heritage resources of the country. There are nearly 1500 marine and 765 freshwater fish species currently reported from India. The Western Ghats and the North- East Region of India, the two biodiversity hot-spots, harbour 583 freshwater fish species alone. However, taxonomic ambiguity exists in several groups of Indian fishes and many are insufficiently identified requiring revalidation.

DNA based approaches to taxon diagnoses can be used to identify and resolve taxonomic ambiguity including discovery of new/cryptic species. We have so far collected 3403 samples covering 656 fish species from freshwater and marine (east and west coasts of India) environments. DNA Barcoding using cytochrome c oxidase I (COI) gene of mt DNA of 304 species including cultivable, ornamental and sport fishes have been completed and 1510 barcodes were generated for the first time in India. The average Kimura two parameter (K2P) distances within species, genus, family and order were 0.50%, 12.50%, 14.93% and 21.10% respectively. The COI sequence information is considered as a reliable means of fish species identification and the technique was successfully employed in forensic identification of whale shark (*Rhincodon typus)* and pomfret (*Pampus chinensis)* as an evidence in the Indian Court of law and Police. The disputed status of *Pristolepis fasciatus, Channa diplogramme, Garra surendranathanii* and *Johnieops vogleri* was revalidated.

We are working towards enhanced application of DNA Barcoding in the identification of fish species banned under Wildlife Act, used in ornamental trade, aquaculture and stocking of reservoirs etc.

ACCURACY OF MORPHOLOGICAL IDENTIFICATION OF LARVAL FISHES

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Due to insufficient morphological characteristics in larval fishes, it is easy to misidentify them and difficult to key to the genus or species level. The identification results from different laboratories are often inconsistent. This experiment aims to find out, by applying DNA barcoding, how inconsistent the identifications are among larval fish taxonomists. One hundred morpho-types of larval fishes were chosen as test specimens. These specimens were delivered, in turn, to five laboratories (A~E) in Taiwan to be identified independently. When all the results were collected, these specimens were then identified using COI barcode. A total of 84 specimens out of 100 were identified to the family (84), genus (73) and species (56) levels based on the COI database currently available. The average accuracy rates of the five laboratories were quite low: 80.4% to the family, 42.9% to the genus, and 14.1% to the species levels. If the results marked as "unidentified" were excluded from calculations, the rates went up to 75.4% and 45.6% for the genus and species levels, respectively. Thus, we suggest that larval fish identification should be more conservative; i.e., when in doubt, it is better to only key to family and not to genus or species level. As to the most misidentified families in our experiment, they were Cheilodactylidae, Malancanthidae, Scomberidae, Scorpaenidae, Sparidae, and Haemulidae. The identifications of the last two families were all wrong. On the other hand, *Mene maculata*, *Microcanthus strigatus* and *Scombrops boops* were all correctly identified to the species level because their larvae have distinct morphology. Nevertheless, barcoding remains one of the best methods to confirm species identification.

BARCODES AND MITOCHONDRIAL PROTEIN EVOLUTION IN FISHES

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It has been shown that several important attributes of complete mitochondrial genomes can be predicted with high accuracy from the DNA barcode sequences alone. These attributes include average nucleotide composition, patterns of strand asymmetry, GC content, and the high frequency of codons that encode hydrophobic amino acids.

This means that DNA barcodes, or other short sequences sampled from a wide taxonomic range, can give a meaningful overview of variations in genome composition long before complete genome sequences become available for many of the sampled taxa.

Such a sentinel approach has been tested in fish for some mitochondrial protein attributes such as rates of evolution and potential selective regimes. The analysis of some 500 mitochondrial genome sequences with broad taxonomic coverage among the fishes showed instances of concerted evolution among protein family members which in turn will allow predictions of the evolution of the Cytochrome Oxidase genes from barcode sequences alone. Some 7000 species of fish are barcoded to date. Large scale analysis with this dataset could confirm first correlations of rate changes detected in this study with life history parameters and physiology.

ADVANCE WITH FISH BARCODES IN MEXICO

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The total fish species in Mexico is about 2200 marine and >500 freshwater species. From these, approximately 500 species have been DNA barcoded by us. In case of freshwater fish, already are barcoded 84 species, 45 genera and 17 families, majority from Yucatan Peninsula and part of the North of Mexico. Barcodes had limited resolution in some flocks of recently evolved species such as the 6 Cyprinodon species from Lake Chichancanab or the Poblana species from the Central Mexican Plateau. For the freshwater species, we had a 93% success; moreover, barcodes highlighted three possible new species. In case of marine fish, success was higher, with 99% correctly identified species.

In total, we have 239 marine fish species barcoded (including adults, larvae and eggs), within 159 genera and 92 families, most of them from the north of the Yucatan Peninsula and the Mexican Caribbean, including some specimens from Belize, Florida and Virgin Islands . One marine species was highlighted as possible new taxa. In several cases larvae unknown could be assigned to species level after match them with the adults, allowing their morphological description. We also confirmed some important nurseries for the first stages of species with economical importance as snappers, groupers, bone fish; and allowed to extend the distribution range for other ones.

At this moment, leading participating institutions in this project are El Colegio de la Frontera Sur-Chetumal, Instituto Politecnico Nacional (IPN) with two branches (Centro de investigación y Estudios Avanzados, CINVESTAV-Merida and Escuela Nacional de Ciencias Biológicas), Icthyology Collection from the Facultad de Ciencias Biológicas (Universidad Autónoma de Nuevo León) and NOAA from USA.

GEOGRAPHICAL SCALE OF SAMPLING AND DNA BARCODING

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While sampling issues have already been debated, the isolated effect of the geographical scale of sampling has not yet been thoroughly tested. Here we present a CO1 dataset (1600 sequences) of an aquatic beetle subfamily, sampled throughout Europe, and use it to test how the geographic scale of sampling affect, i) the intraspecific variation of species, ii) the genetic distance to closest heterospecific, iii) species monophyly, and, iv) we use randomizations to tackle the question of how many samples are enough.

Geographical scale of sampling was significant and had a high explanatory power (r2=0.7) on the intraspecific variation, which exceeded 1% in 69% of species with >10 sampled individuals (N=29). Distance to closest heterospecific showed a significant decrease with increasing geographical scale of sampling. The average genetic distance dropped from >7% of all samples within 1km, to <3.5% of all samples up to >6000 km apart. The proportion of monophyletic species was an increasing function through regionally (5%), nationally (13%), internationally (22%) and continentally (37%) restricted subsets of the data. With randomizations we show that a sampling scheme that maximizes the geographic coverage does better than random, but still underestimates the intraspecific variation at surprisingly large sample sizes.

The results have wide-ranging implications on the global barcoding effort. First, given the present Linnaean taxonomy and if representative of the European diversity, 30-40% of the fauna is relatively young, poorly differentiated by CO1, and species level identification can be ambiguous. Second it emphasizes the crucial importance of wide geographical sampling. Finally we predict that, a globally queried database may often result in ambiguous conclusions, but the allopatric nature of many sister species pairs gives advantage to a geographically restricted query-approach if the region is adequately sampled or faunistic databases are co-linked.

DNA BARCODING OF SHORE FLIES FROM GREAT SALT LAKE

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Great Salt Lake, in northern Utah, is one of the largest lakes in the United States, with a total surface area of 4400 square kilometers. Shore flies (Ephydridae) are among the most important components of the Great Salt Lake ecosystem, removing an estimated 90 million kg of organic matter from the benthos of the lake and serving as an important food source for both resident and migratory bird populations. During peak concentrations, in the summer months, the numbers of flies are astounding, with estimates of nearly a billion flies per kilometer of lakeshore. In spite of their essential role in this ecosystem there are few published studies on shore fly identity and diversity. Reports from the state of Utah list two principle species, Ephydra gracilis and Cirrula hians. However, at least six other species have been collected, some of which are difficult to distinguish morphologically. Species can be easily differentiated based on a size polymorphism of the internal transcribed spacer region (ITS-1), which is found between the 18S and 5.8S nuclear ribosomal RNA genes. This study compares the ability of the ITS-1 polymorphism and DNA barcoding using the mitochondrial cytochrome oxidase I gene to identify shore flies. Data on intraspecific and interspecific sequence variation for both loci are discussed for selected species.

IDENTIFYING CRYPTIC INVASIVE ANTS IN THE SOUTHWEST INDIAN OCEAN

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Ecosystems become increasingly vulnerable to nonnative species invasion as fragmentation, anthropogenic disturbance and numbers of introductions intensify. Insular and fragmented systems such as the islands of the Southwest Indian Ocean (SWIO) are particularly vulnerable to invasives such as ants, which cause a wide range of drastic community changes. The first step in understanding the threat posed requires mapping the current extent of ant invasions in the SWIO. Using traditional morphological approaches inn a hyperdiverse tropical ecosystem, identifying native versus introduced species is no easy task.

As part of an ongoing effort to inventory and DNA barcode the ant fauna of the region, we investigated the use of DNA barcode data to indicate possible introduced species to the region. When compared across the island system, species known to be introduced showed lower sequence divergence values than native congeners. An analysis across all ants in the region revealed introduced species from both unnamed and named taxa that were previously thought to be endemic to the region. Overall, the results show the utility of DNA barcode data to highlight

introduced species in large inventory programs and the feasibility of developing a worldwide identification and monitoring system for invasive ants.

INFERRING BIODIVERSITY PATTERNS AND LIFE-HISTORY TRAITS IN RAY SPIDERS (ARANEAE, THERIDISOMATIDAE)

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DNA barcoding promises to revolutionize biodiversity studies by allowing identification of all life stages and facilitating species discovery. In this contribution, we evaluate the advantages of DNA barcoding techniques to assess alfa and beta diversity in a biodiversity hotspot, the clouds forest of Panama. The orbicularian spider family Theridiosomatidae is the sister groups of all the remaining "Symphytognathoids", is very diverse, with 12 genera and 77 species distributed in four subfamilies.

Theridiosomatidae is a good candidate to evaluate the benefits of the use of barcoding approaches to investigate biodiversity patterns. The taxonomy is relatively well known at the genus level. One of the most distinctive traits of the family are the egg-sacs. They had different kinds of shapes: cubical, spheroidal, pear-shaped, or fluted. Some genera have been documented to build particular types of egg-sacs, others built similar ones, and some others more than one type. In addition, we used barcode techniques to link egg-sac types to particular species to gain insight on the usefulness of egg-sac shape for bioinventoring purposes.

The collected samples were sorted at the morphospecies level, with 24 morphospecies, and nine egg-sacs types. Morphotypes from each morphospecies/species and one egg-sac from every type were selected to be imaged. The 5'-cox1 fragment for five specimens per species per locality were sequenced, in total 241 specimens and 41 egg-sacs.

Neighbor-joining results, supported by bootstrap values, showed 100% matching between our previously selection of morphotypes belonging to the same morphospecies. Egg-sacs types corresponded with adult's morphospecies of the same genus, and from the same locality. In addition, an unexpected preliminary result of our study was the finding of wasp parasites (Ichneumonidae, Hymenoptera) in some of the analyzed egg-sacs. DNA barcodes allowed us to identify them. Alfa and beta diversity is discussed.

DEGREE OF 28S rDNA DIVERGENCE IN COSTA RICAN BARCODED LEPIDOPTERA

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Animal DNA barcoding with mitochondrial cytochrome c oxidase 1 (CO1) is based on short species-specific sequences. COI has the advantage that there are multiple copies in most cells and it can be "mini-barcoded", both of which allow for processing samples with degraded DNA. CO1 barcodes provide high species-level resolution (i.e. 95%) in groups such as birds, fish and Lepidoptera. However, there are circumstances where corroborative nuclear sequences would lend credence to whether barcode clusters separated by only a small phylogenetic distance and not correlated with non-sequence traits are of the same species.

We examined the appropriateness of ribosomal DNA (rDNA) for this end. Specifically, we compared results of barcoding with the D2 expansion portion of the 28S subunit to that of barcoding with CO1 sequences derived from the same individuals in 620 Lepidoptera species from Area de Conservacion Guanacaste in northwestern Costa Rica. This marker, like mtDNA, is appropriate for degraded samples because there are several hundred copies per nucleus and it offers the possibility of capturing short amplicons from conserved regions. We chose stem-rich regions that were alignable across all samples and derived a NJ tree using gaps as a fifth character state. Intra-generic variation (measured as the pairwise percentage differences) is higher in CO1 than 28S (mean of 12% vs. 2%, respectively), while inter-generic distance is greatest in 28S (20% vs. 13%). Overall, 88% of species contained unique 28S sequences. In many groups, the 28S locus appears to provide better support for genus-level monophyly (based on conventional morphologically-derived assignments) than did CO1, although the difficulty of alignment at loop regions confounds robust phylogenetic signals at deeper levels. We suggest that the D2 region of 28S can be used as a powerful marker for species diagnosis in parallel with CO1, especially when a barcode cannot be obtained.

MOLECULAR TAXONOMY OF GROUND BEETLES IN CENTRAL EUROPE:
A MULTI-MARKER APPROACH

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The valid identification of species represents a pivotal component for documenting large-scale biodiversity studies and conservation planning. However, routine identification of many species can be difficult and time-consuming, often requiring highly specialized knowledge and representing a limiting factor in biodiversity and ecological studies. Especially the identification of larval stages of highly diverse taxa using classical morphological methods constitutes an impossible

task. In this context, a DNA-based taxonomy analysing a vast number of specimens has been proposed to ensure fast and accurate species identification in biodiversity research in the past few years. Furthermore, the genetic cohesiveness and taxonomic integrity of species with widespread or disjunctive geographical distributions can be evaluated using DNA sequence data. In our project we study and compare the molecular taxonomic quality of different markers for species identification of Middle European Carabidae, using the classical mitochondrial barcode marker CO1 but also species-variable and informative short nuclear gene fragments. As opposed to mitochondrial gene fragments, the analysis of appropriate nuclear gene fragments (with a length of 200 to 500 base pairs) will avoid well-known problems of mitochondrial data, namely introgression events, ancient polymorphisms, or effects of *Wolbachia* infections.

DNA BARCODING AND THE PHYLOGEOGRAPHY OF THE BLACK FLY *PROSIMULIUM TRAVISI* (DIPTERA: SIMULIIDAE)

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DNA barcoding has gained increased prominence as a tool for species identification; it has also proven useful in more applied fields, such as ecology, conservation and food-product validation. In this study we present evidence that the barcoding gene has utility for inferring phylogeographic patterns in the Nearctic black fly *Prosimulium travisi* Stone. This species is widely distributed in the Cordillera of western North America, from Alaska and the Yukon Territories to California and New Mexico. The northern half of the present-day distribution of *P. travisi* was largely covered by continental ice during the last (Wisconsinan) glaciation, and populations now inhabiting this terrain were the products of postglacial immigration from refugia areas. As mitochondrial genes have potential to reveal phylogeographic structure, we investigated whether the DNA barcoding gene could (a) reveal the source areas (i.e., refugia) of *P. travisi* and (b) elucidate the routes by which founding populations recolonized previously glaciated terrain.

Three hundred and thirteen individuals representing 56 populations were sampled across the entire range of *P. travisi*. Standard population genetic analyses were performed and intraspecific phylogenies were constructed in order to reveal phylogeographic structure. The results suggest that northern cordilleran populations were derived from postglacial immigration from Beringia, a northwestern coastal refugium and a southern cordilleran refugium. Postglacial migratory routes were established, and areas of secondary contact among founding populations were identified. Additionally, a cryptic species was identified in the high mountains of Colorado, perhaps the product of an earlier glacial cycle.

Our results are congruent with phylogeographic studies of other widely distributed cordilleran organisms. We conclude that the DNA barcoding gene has utility for revealing biogeographical patterns in widely distributed and comprehensively sampled species.

DNA BARCODING LEPIDOPTERA: WHAT BEYOND TAXONOMY?

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Lepidoptera have served as a model-group to test the effectiveness and utility of DNA barcoding. Today, these insects are the most heavily sampled organisms, both numerically (>300K barcodes in BOLD), taxonomically (>35K species), and geographically (samples from 190 countries). The value of DNA barcodes for taxonomic revisions has been demonstrated in different families, building support from the global community of lepidopterists. However, DNA barcode data have much broader implications for biodiversity studies. In this presentation, we discuss case studies revealing how DNA barcodes, by refining our knowledge of biodiversity, contribute to advances in evolutionary biology, phylogeography, conservation biology, and ecology. We also show the new vision of Lepidopteran biodiversity offered by DNA barcodes when considering biogeographic patterns. Using examples from two large families of Lepidoptera (Saturniidae, Sphingidae) for which the world fauna (4000 species) is close to being barcoded – we highlight some interesting cases of shared or distinct geographic patterns of genetic variation, as well as some newly revealed patterns of endemism. The growing resource represented by DNA barcodes of Lepidoptera is unique and we stress the need for a comparative and multidisciplinary approach to further analyze these data.

DNA BARCODING OF ARCHIVAL LEPIDOPTERA SPECIMENS

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Natural history museums hold vast numbers of type specimens, many collected in the 18th and 19th Centuries, whose sequence characterization is critical for the validation of current taxonomic assignments. Despite their high scientific value, most museum specimens are inaccessible to conventional DNA barcoding analysis because the degradation of their DNA impedes PCR amplification with standard protocols. To overcome this difficulty, we present a new protocol for amplification which targets six short, overlapping fragments of the standard DNA barcode region.

Primer sets were developed based on sequence attributes of the family Sphingidae, but were subsequently tested on a diversity of museum samples. Partial or total recovery of the DNA barcode was obtained from specimens belonging to 13 families in 7 superfamilies of Lepidoptera, with a success rate higher than 70%. The oldest sample sequenced was the original type specimen of a noctuid described in 1788. We emphasize the importance of genetic data gained through such analysis by presenting selected examples of taxonomic revisions enabled by the barcode analysis of type specimens. We also emphasize the importance of analyzing taxa which are now believed extinct or that have never been re-collected. Although special precautions are needed to avoid contamination, this new protocol can be integrated in a high-throughput automated workflow.

TESTING A SHORT NUCLEAR BARCODE FOR INFERRING STAPHYLINID BEETLE DIVERSITY IN AN AFRICAN RAINFOREST

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DNA barcoding has been suggested as an alternative to traditional morphology for describing patterns in biodiversity for hyperdiverse but morphologically little distinct animal communities, e.g. beetles of tropical rainforests. However, the capability of single markers to capture all species of standardized environmental samples and to reveal biodiversity patterns has not been tested. Here a short nuclear marker (28S rDNA: D2, ~180 bp) is used along with a morphological approach to describe the diversity of staphylinid beetles of a Congo-Guinean rainforest in Kenya and to compare biodiversity patterns between primary and secondary forests. The value of secondary forests for the conservation of primary forest animal communities is actually a highly debated topic in conservation science. Beetles were collected in a standardized experimental design using pitfall traps along six 200m transects (three per habitat). DNA of one individual of each morphotype in each trap was extracted from 2-3 legs, while the remaining body was mounted for morphological analysis. Using the D2 marker it was possible to amplify and sequence DNA of 99.06% of all individuals (421 specimens), including even DNA from smallest specimens of only ~1.5mm length. In total 76 molecular operational taxonomic units (MOTU) in contrast to 70 morphotypes were found. Both approaches revealed highly similar biodiversity patterns, with species diversity being equal in both habitats, but strongly divergent species communities between habitats. Therefore, both the molecular and morphological approach suggest that secondary forest may not be adequate for the conservation of many primary forest beetle species. The study shows that molecular markers can provide an alternative to morphological methods for studying the biodiversity of hyperdiverse insect taxa. The efficient amplification of the D2 marker and its capability to delimit meaningful units allow its use in future molecular studies on biodiversity.

PERFORMANCE OF DNA BARCODING FOR INSECT IDENTIFICATION

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Previous studies on insect DNA barcoding provide contradictory results and suggest not consistent performances across orders. This work aims at providing a general evaluation of insect DNA barcoding and "mini-barcoding" by performing simulations on a large database of 15,948 DNA barcodes. We compared the proportions of correctly identified queries across a) six insect orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Orthoptera), b) four identification criteria (Best Match: BM; Best Close Match: BCM; All Species Barcodes: ASB; tree-based identification: NJT), and c) reference databases with different taxon coverage (100, 500, 1,000, 1,500 and 1,995 insect species).

Analysis of variance revealed highly significant differences among ID criteria and insect orders. *A posteriori* comparisons of means showed that NJT had always a significantly lower identification success (NJT = 0.656, S.D. = 0.118) compared to both BM and BCM (BM = 0.948, S.D. = 0.026; BCM = 0.946, S.D. = 0.031). NJT showed significant variations among orders, with the highest proportion of correctly identified queries in Hymenoptera and Orthoptera and the lowest in Diptera. Conversely, the proportions of correct matches of BM and BCM were consistent across orders but a progressive increase in false identification was observed when larger reference databases were used.

Regardless the relatively low proportion of Type I errors (misidentification of queries which are represented in the reference database) of BM and BCM, the lack of reference DNA barcodes for 98% of the known insect species implies that insect DNA barcoding can be heavily biased by Type II errors (misidentification of queries without conspecifics in the database). The detrimental effects of Type II errors could be circumvented if insect DNA barcoding is used to verify the lack of correspondence between a query and a list of properly referenced target species (*e.g.* insect pests). This "negative identification" would only be subjected to Type I errors and could be profitably adopted in insect quarantine procedures.

TRICHOPTERA BARCODE OF LIFE: PROBING CADDISFLY DIVERSITY WITH DNA BARCODES

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Trichoptera are one of the most diverse insect groups found in freshwater habitats. These insects, especially their larvae, are particularly useful as indicators of water quality and have been widely adopted in monitoring programs. However, species identification, particularly of larvae and females has been difficult. The Trichoptera Barcode of Life campaign aims to overcome this barrier

by building a barcode reference library for all caddisfly species, including more than 13,000 species worldwide. Since its launch in mid-2007, 13K barcode sequences have been collected from more than 1.7K species, representing approximately 13% of the global fauna. Furthermore, representatives from 44 of 46 families, and from more than half of the 610 described genera have seen barcode analysis. The broad taxonomic coverage in barcode reference library provides the first insight of the Trichoptera diversity from a molecular respect. Resulting from a large-scale international collaboration, numerous taxonomic discoveries have already been made. Probable cryptic species have been revealed in many groups of caddisflies. DNA barcodes are also providing molecular evidence in support of overlooked synonymies and for the need to re-evaluate some past species 'amalgamations'. TrichopteraBOL provides various support to collaborators, such as free DNA sequencing, sharing of DNA extracts, and use of the Barcode of Life Data System (BOLD) as a data repository and analytical workbench. TrichopteraBOL is also fostering a large-scale, collaborative investigation on the global fauna. This presentation will summarize the contributions of this project to Trichopteran taxonomy, systematics, life-stage association, and biomonitoring.

INTERNATIONAL ECOSTATIONS JOURNAL: BioIP MANAGEMENT SOLUTION

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Research stations, governments, and other institutions responsible for conducting and managing field biology are processing a growing volume of applications (e.g., for permits) and reports (often a requirement of permits). At the same time, the success of DNA barcoding, and the increasing use of molecular tools in ecological research, is generating more requests for the transfer of biological materials (macro and microbial) from field sites to labs and museums, sometimes in other countries. Such activities raise concerns about the use of genetic resources, an issue addressed by the Convention on Biological Diversity (CBD) through its Ad Hoc Working Group on Access and Benefit-sharing. Whether the intent of the research is commercial or non-commercial, all parties benefit from efficient and transparent administrative systems that (a) document Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) for the research, (b) enable materials to be tracked as they cascade through the research value-chain, and (c) ensure the terms of use follow the materials downstream (e.g., through Material Transfer Agreements, MTAs). Here we present an online solution that we have developed as part of the Moorea Biocode Project, an international collaboration conducting an All Taxa Biotic Inventory of a tropical island (coral reef, freshwater, and terrestrial ecosystems) including DNA barcoding of all species. The management system is based on an e-journal platform; it aims to reduce the bureaucratic burden on scientists, research stations, and government agencies, while ensuring traceability of the materials and promoting the accessibility of research results for all stakeholders.

BARFROST – A NEW PROJECT FOR RECONSTRUCTING PAST ECOSYSTEMS BY BARCODING DNA FROM PERMAFROST

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 Recent advances in ultra-high-throughput DNA sequencing technology and in species identification ('barcoding') tools based on ancient DNA preserved in permafrost soils are opening up a novel research avenue for paleoecological reconstruction. In this project, we are using these novel approaches to analyse past Arctic biodiversity from ancient DNA preserved in permafrost soils. We are developing new DNA barcoding markers for several groups of organisms (bryophytes,

fungi, insects, springtails, vertebrates) that are short enough to amplify from degraded DNA. These markers will be sequenced in modern DNA to construct taxonomic reference databases for ecologically important arctic species and thereafter used for species identification by pyrosequencing environmental DNA from dated circumarctic permafrost core samples. The new data obtained for these organisms, along with complementary data for vascular plants, will be used for reconstructions of past ecosystems. Preliminary results obtained for vascular plants suggest that the reconstructions will be considerably improved compared to traditional fossil analyses. The results can therefore have immediate bearing on central ecological issues such as past species turnover dynamics in assemblages, niche stability in time and space, and backward testing of predictive species distribution models.

THE NEON FUNDAMENTAL SENTINEL UNIT: ORGANISMAL MEASUREMENTS AND DNA BARCODING IN A NATIONAL NETWORK

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The National Ecological Observatory Network (NEON) is a national-scale research platform for analyzing and understanding the impacts of climate change, land-use change, and invasive species on ecology. NEON features sensor networks and experiments, linked by advanced cyberinfrastructure to record and archive ecological data for at least 30 years. Using standardized protocols and an open data policy, NEON will gather essential data for developing the scientific understanding and theory required to manage ecological challenges. NEON data will be gathered from the level of the gene and organism to populations and communities, which shall be extrapolated to the continental scale. The scaling strategy requires a mixture of human and instrumental measurements. The Fundamental Sentinel Unit (FSU) is responsible for the field observations and analyses of biological specimens that will provide data on biodiversity, population dynamics, productivity, phenology, infectious disease, biogeochemistry and ecohydrology. The observatory will track patterns in communities including: microbes, plants, algae, insects, aquatic invertebrates, birds, fish and small mammals. DNA barcode libraries will be developed for some of these sentinel organisms in order to facilitate species identification. The FSU design is intended to reflect the best available science, be compatible with existing programs, be flexible across a range of environmental gradients, and facilitate research across a broad range of areas. The data collected by the FSU will enable research across several disciplines. Here we present a few examples of the type of research NEON will enable using FSU data including infectious disease, invasive species, phenology, and ecohydrology.

GENOMAPS: DNA BARCODING APPLIED TO A LARGE-SCALE BIODIVERSITY MONITORING IN SOUTH AMERICA

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Biodiversity surveys are usually accomplished using species lists (presence/absence data). However, in order to detect and prevent biodiversity losses we need to know not only where species are, but how locally abundant they are, and when they were there: declines cannot be observed with presence-absence data from a single point in time. To complicate things further, existing biodiversity collections employ highly biased and sporadic sampling and remain largely un-integrated, particularly in the tropics, thereby limiting access to the basic information needed for developing systematic conservation monitoring in the world's most biodiverse regions. The solution implies identifying and defining biodiversity units, and then monitoring their spatio-temporal trends. To define measurable units, we must integrate new molecular technologies with existing and new collections in a biological systematics framework. To monitor spatio-temporal trends in these units' abundances, we must implement a sampling design that acquires maximum information for minimum effort. GenoMaps will contribute to both of these goals by employing DNA barcoding and other molecular approaches as a component of a major new sampling effort, NeoMaps, to establish a systematic baseline of the distribution and abundance of major arthropod taxa over a large tropical region. First, to define units, GenoMaps will make use of the more than 30,000 butterflies already collected and morphologically identified by NeoMaps, to construct a database of reference barcode sequences. New taxonomic units will be defined where necessary based on a combined molecular/morphological approach, so that the reference database is continuously updated. Then, to monitor spatio-temporal trends, we will proceed with mass molecular identification of specimens, to create a combined distribution/ relative abundance dataset for all taxa considered. Mass identification will be repeated every 5 years in order to detect changes through time, freeing limited taxonomic expertise from routine identification to concentrate on complex systematic challenges. In the future, GenoMaps will expand to include other biodiversity indicators, such as dung beetles, and to disease vectors, such as mosquitoes and freshwater snails.

APPLICATION OF DNA BUSHMEAT BARCODING IN PROSECUTION OF WILDLIFE CRIMES IN EAST AFRICA

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Uganda, Kenya and Tanzania apply common wealth legal system. The three countries have the same legal system with the legal precedents (judgments) pronounced in one state binding on others. This paper assessed the application of DNA barcoding of bushmeat in prosecution of wildlife crimes in Kenya ,Uganda and Tanzania. The study shows that in courts in the study areas, application of scientific technology or opinion to prove cases are inadequate.The study also examine the laws in the three countries to ascertain the legal readiness to embrace bushmeat barcoding as a scientific tool in the prosecution of wildlife crimes in the region.

The study shows that in Uganda and Kenya the Evidence Act has placed the burden of proof in criminal cases , wildlife crimes inclusive   on the state/ prosecution to prove that the species poached is wildlife under section 104 of the Uganda Evidence Act The burden of proving any fact necessary to be proved in order to enable any person to give evidence of any other fact is on the person who wishes to give that evidence. and the required standard of proof is proof beyond reasonable doubt.

Under section 105(b) of the Uganda Evidence Act the person accused shall be entitled to be acquitted of the offence with which he or she is charged if the court is satisfied that the evidence given by either the prosecution or the defense creates a reasonable doubt as to the guilt of the accused person in respect of that offence this provided for this was further illustrated in the case of **Uganda Vs Dick Ojok [1992-93] HCB 54**, where court held that in all criminal cases the duty of proving the guilt of the accused always lies on the prosecution and that duty does not shift to the accused person and the standarad by which the prosecution must prove the guilt of the accused is proof beyond reasonable doubt except in a few statutory cases. Any legally acceptable doubt raised in court is always resolved to the benefit of the accused.

This impacts on the prosecution of wildlife crime because in the absence of visible morphological features like trophies, hair , hooves , smoked or sundried ,bushmeat from antelopes are impossible to differentiate from goats meat , buffaloes from beef , warthogs and wild pigs from pork. In the absence of scientific proof courts in east Africa have always ruled that prosecution did not prove their case beyond reasonable doubt and the suspects are acquitted.

The absence of Bushmeat DNA barcoding in prosecution has motivated the habitual wildlife criminals, commercial wildlife poachers in east Africa to poach while knowing that in the absence of visible morphological features the prosecution will miserably fail to prove their case beyond reasonale doubt.the provision of the law has frustrted the efforts of the wildlife crime law enforcement rangers who usally take their time going through a costly investigation processes.

whereas in Tanzania in an attempt to improve on the prosecution of wildlife crime, the state have shifted the legal burden of proof on the accused to prove that the species found in his or her possession is not wildlife or that it was acquired legally .The study also examined the professional capacity of magistrates, prosecutors and law enforcement rangers in the use of DNA bushmeat barcoding in wildlife crime prosecution.The study finally recommended training of magistrates,prosecutors and police on use of DNA bushmeat barcoding in wildlife law enforcement,and development of wildlife crime prosecutors manual.

APPLICATIONS OF DNA BARCODES FOR STUDIES OF ANTARCTIC KRILL: SS-PCR AND MITOCHONDRIAL SNPs

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Mitochondrial genes used for DNA barcoding exhibit patterns of intra- and interspecific variation that are advantageous for diverse applications in studies of marine zooplankton. The barcode region of cytochrome oxidase I (COI) clearly discriminated three species of Antarctic krill (Euphausia superba, E. crystallorophyas and Thyasonessa macrura) that require special expertise to reliably identify as larvae and juveniles. Low levels (1-2%) of variation within species and high levels (20-25%) of variation between the species allowed the design of a rapid, inexpensive, and accurate protocol for species identification using a multiplexed, competitive species-specific PCR (SS-PCR) reaction. The protocol is robust and reliable when used by inexperienced personnel and on any PCR machine.

Analysis of DNA sequence variation for another protein-coding mitochondrial gene, cytochrome B (CytB), revealed high levels of variation at many single nucleotide polymorphism (SNP) sites. Four SNPs, all four-fold degenerate, silent substitutions at third codon positions, were selected as characters for a study of population genetic variation of the Southern Ocean krill, Euphausia superba. Collections were made at the Southern Ocean U.S. GLOBEC study site in the Western Antarctic Peninsula (WAP) region. SNPs were detected using the ABI SNaPshot kit, with multiplexed reactions to simultaneously detect alleles at all sites. Analysis of SNP allele frequencies revealed small-scale time/space variation among samples, with significant differentiation between juvenile krill of the same life stage from different samples. This study revealed the power of mitochondrial barcode SNPs as markers of small-scale population genetic variation in natural populations of high gene-flow species, such as marine zooplankton.

MOLECULAR TAXONOMY OF PUTREFIED CETACEANS - A CASE STUDY FROM THE SOUTHWEST COAST OF INDIA

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Cetaceans (whales, dolphins and porpoises), the charismatic marine mammals protected under Indian Wildlife (Protection) Act, stranded along the Indian coasts are often not properly identified due to the lack of taxonomic expertise and poor quality of the specimens. This paper describes the taxonomy of two putrefied carcasses of cetaceans without key taxonomic structures washed

ashore at Edayar (08° 25' N lat.; 76° 57' E long.), Thiruvananthapuram district, southwest coast of India, using sequencing of mitochondrial genes such as 12S, 16S, cytocohrome oxidase (CO) I and cytochrome b. The tissue samples collected from cetaceans were processed for the extraction of DNA using standard protocols and amplified with universal primer for COI and then sequenced. DNA sequences obtained were aligned with known COI sequences of the whale sequences using bl2seq (NCBI). Phylogenetic affiliation of the COI mitochondrial gene sequences was determined using neighbour-joining tree of Kimura 2-parameter distance model.

Sequence similarity search done for COI mitochondrial gene with all entries in the GeneBank using BLAST showed 100% sequence similarity with Bryde's whale (*Balaenoptera edeni* Anderson) (Family: Balaenopteridae) for the first specimen and 97% sequence similarity with finless porpoise [*Neophocaena phocaenoides* (Cuvier)] (Family: Phocoenidae) for the second specimen. Phylogenetic position of both the samples for 16S rRNA gene sequenced also confirmed the identity of the specimen.

The present work is the second report of *B. edeni* from southwest coast of India since 1800, and comarative studies showed that the populations inhabiting seas around India could be the smaller form of species in 'Bryde's Whale Complex' and we propose that the common name of the whale inhabiting the seas around India could be considered as Eden's whale rather than as Bryde's whale. Our phylogenetic studies also corroborate the contention of related studies that *B. edeni* constitutes a sister taxon to *B. brydei*. The study propose that though osteological details would provide clues for identification of marine mammals, COI sequences can be used as a reliable method for assigning species status to marine mammals stranded in putrefied condition and for documenting the unknown marine mammals that often form bycatch of modern fishing gears.

GLOBAL PHYLOGEOGRAPHIES OF THE PLANKTONIC COPEPOD CLAUSOCALANUS BASED ON DNA BARCODES

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The DNA barcode region of mitochondrial cytochrome oxidase I (COI) exhibits low levels of variation (1% to 4%) within species of many planktonic copepods (Arthropoda, Crustacea, Copepoda). For globally-distributed species, patterns of COI variation may elucidate patterns and pathways of gene flow, inform phylogeographic and population genetic studies, test hypotheses of genetic cohesion, and reveal taxomically significant geographic variation and cryptic species. This comparative global phylogeographic study seeks to examine variation of the COI barcode region within species of the copepod genus Clausocalanus, and to relate biogeographical distributions and patterns of gene flow to the geological history of the oceans.

We show that circumglobally-distributed Clausocalanus arcuicornis has high COI haplotype diversity, with variance concentrated within populations and shared haplotypes between Atlantic and Pacific Ocean populations. This suggests a panmictic population across this species' extensive geographic range, with sufficient gene flow throughout the cosmopolitan distribution (despite vast distances and geological and oceanographic barriers to gene flow) to maintain genetic cohesion. In contrast, biantitropical Clausocalanus lividus exhibits lower levels of haplotype diversity, with variance concentrated between populations, no shared haplotypes between Atlantic and Pacific populations, and clear differentiation among populations in different ocean basins. Several approaches to analysis (e.g., population genetic and phylogenetic) and visualization (e.g., cluster, spatial autocorrelation, GoogleEarth) are used to examine hypotheses of the role of ocean circulation and the geological evolution of the ocean basins in driving the observed differences between the species

DNA BARCODING OF MARINE ZOOPLANKTON: CURRENT STATUS AND APPLICATIONS FOR ECOSYSTEM MONITORING

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The Census of Marine Zooplankton (CMarZ) is working toward a global database of gold standard DNA barcodes for the ~7,000 described species of marine holozooplankton. We report here on progress toward a "gold standard" barcode database for zooplankton, including DNA sequences for the barcode region of mitochondrial cytochrome oxidase subunit I (mtCOI) for planktonic representatives of15 animal phyla. Samples have been collected from every ocean basin and from surface to bottom (>5,000 meters). Living specimens are identified to species immediately after collection; barcoding has been carried out in ship-board DNA sequencing laboratories. We describe novel approaches to data analysis, visualization of species diversity, and identification and quantification of known species using barcodes. Current applications include revealing cryptic species, describing biogeographical distributions, and discovering new species. Future applications include rapid characterization of species diversity in unsorted samples using environmental barcoding (community metagenetics) for ecosystem monitoring.

MOLECULAR SYSTEMATICS OF PRAWNS UNDER THE FAMILY PENAEIDAE OF INDIAN COAST

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Majority of the world's commercially important shrimp species are included under the family Penaeidae. Twenty six valid genera have been reported under this family, of which 13 genera are represented in Indian waters. For the present study, 16 species belonging to 5 genera viz. *Fenneropenaeus*; *Metapenaeus*; *Parapenaeopsis*; *Penaeus* and *Trachypenaeus* collected from three locations on the west coast (Gujarat, Maharashtra, and Kerala) and two locations from east coast (Andhra Pradesh and Tamil Nadu) of India have been considered. All the specimens were identified using the phenotypic characters. The phylogenetic relationships was inferred using the sequence data from 650 bp region of cytochrome c oxidase subunit I (COI) and 510 bp region of 16S rRNA genes. The morphologies of two genera of shrmps *Fenneropenaeus* and *Penaeus* are very similar, which can be differentiated only by the presence and absence of hepatic ridge, the genetic analysis of the present study revealed high divergence in genes, indicating that they belong to different genera. Genetic variation was observed among the east and west populations, suggesting absence in mixing of both the populations. The present study highlights the genetic diversity in penaeid shrimps and the use of mitochondrial markers as tool to differentiate the species.

MARINE INVERTEBRATES AND THE "BARCODE FACTORY" AT THE MUSEUM NATIONAL D'HISTOIRE NATURELLE

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For the last 25 years, the bathyal areas of South-Western Pacific have been intensively sampled by the Museum National d'Histoire Naturelle (MNHN) and the Institut de Recherche pour le Developpement (IRD). Over 11 localities, about 4000 stations have been trawled or dredged at depths ranging from 100 to 3700 meters and impressive collections of benthic invertebrates have thus been gathered. An important part of this material has been fixed in alcoohol. Moreover, since 2005 our sampling protocols have been especially designed for molecular barcoding. These collections are thus a first-choice material to assess the diversity of marine invertebrates in the South-Western Pacific and to built a reference database. This database will serve as a framework for taxonomic expertise and further species descriptions in a integrative framework including molecular data.

As leading laboratory of the Marine Barcoding of Life project, the MNHN has been involved in this task for now almost one year. A new database has been developed to link molecular information

to other data classically recorded for every analysed specimen such as photos, geographical coordinates, depth, substrate, and other field data and to export all of these to the BOLD System. Both in the field and in the lab, the process of data acquisition has been split into simple tasks from specimen sorting and processing to COI mtDNA sequencing. Here will be presented the main elements of this "Barcode factory", including the sampling plan, the database and the process of data acquisition.

STANDARDIZED SAMPLING AND DNA BARCODING FOR ASSESSING CORAL REEF BIODIVERSITY

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Coral reefs are among the most diverse ecosystems on earth and also one of the most threatened. In this context, understanding patterns of biodiversity distribution is essential to conservation strategies.

Traditionally well-studied reef-associated macrofauna such as corals and fishes have often been used to achieve rapid strategic biodiversity assessments. However, it is questionable whether the use of surrogate groups for biodiversity assessment on coral reefs reflects general patterns of diversity among all organisms and diversity data on the most numerous reef-associated cryptofauna remains scarce and scattered. Because of time constraints, tremendous scientific effort required and lack of taxonomic expertise, exhaustive inventories of this extremely diverse, small-bodied fauna still remain impractical.

In this geographically extensive study, we provide new biodiversity estimates of the reef-associated cryptofauna using an innovative new method. DNA barcodes are generated for the entire crustacean community extracted from standardized samples of both natural reef environments and artificial structure units. Overall, more than two thousand crustaceans DNA barcodes from major crustacean groups have been generated from sites in the Pacific Ocean (The Line Islands, French Polynesia, Northwestern Hawaiian Islands, Great Barrier Reef Lizard and Heron Island) and Indian Ocean (Western Australia). These barcodes, assigned to Operational Taxonomic Units using different levels of sequence divergence, are used to estimate total biodiversity using statistical diversity estimators and predict the number of species yet to be discovered. Results reveal the high prevalence of low abundance species and high species turnover between localities and across reef habitats.

We demonstrate that a strategic sampling regime associated with less labor-intensive approaches (i.e., DNA-based technologies) can narrow considerably the uncertainty of estimates of reef biodiversity.

MARINE CRUSTACEANS IDENTIFIED BY DNA BARCODES

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Crustaceans are one of the most diverse groups of metazoans from a morphological and ecological point of view. In the marine realm, they are an important component of food webs and some species are targeted by major fisheries. As a taxonomically difficult group, often requiring the help of highly-trained taxonomists, crustaceans would greatly benefit from DNA barcoding. A database of barcodes will assist in the identification of specimens during all their life stages, which is especially useful in the case of heteromorphic species. Practical applications include detection of marine invaders spreading as larvae or stock assessment of commercial species based on larval abundances. Moreover, such data will flag cases of cryptic speciation. Here we present the progress in barcoding marine crustaceans from Atlantic Canada based on cytochrome c oxidase 1 sequences. Our results show that DNA sequences usually group in clusters corresponding to known morphological species, except for cases of potential cryptic species (3.5-19.5% intraspecific variation). Of the two main crustacean groups targeted here, amphipods had more cases of deep intraspecific divergence than decapods. This finding might be explained by a lesser potential for dispersal in amphipods (no larval phase) versus decapods (larval phase), leading to different speciation rates. Consequently, DNA barcodes from large-scale studies may be useful not only for species identification but also for inferring evolutionary patterns in different groups of crustaceans.

THE INTELLIGENT OBSERVER: USING THE BARCODE OF LIFE DATABASE TO INVESTIGATE THE ROLE OF THE OXPHOS PATHWAY IN POST-ZYGOTIC ISOLATION

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DNA barcoding is an automated system for indexing and retrieving taxonomic information which uses a standardized gene region as a proxy for recognizing species boundaries. The gene region used to identify animal life is mitochondrial cytochrome c oxidase I (COI). Already barcode records for COI have grown to include several tens of thousands of species and expand rapidly as campaigns to barcode large blocks of animal life continue to be launched. In addition to serving as an essential backbone for delivering species identifications, we would like to emphasize that the COI barcode database also represents the most comprehensive collection of standardized population-level genetic data and provides an invaluable resource for a priori hypothesis generation within several evolutionary contexts. For example, functional incompatibility between mitochondrial- and nuclear-encoded components of the co-adapted gene complex responsible for oxidative phosphorylation (OXPHOS) is increasingly becoming recognized as important cause of

post-zygotic isolation. The barcode database provides measures of intra-specific variation from individuals chosen to represent the distributional geographic range of each species and is perfectly suited for locating source populations of varying genetic divergence for testing fitness effects of inter-population hybrid crosses. The framework readily provides the opportunity to initiate parallel programs to quantify the role and frequency of the OXPHOS pathway in contributing to outbreeding depression for a diversity of animal life. Such an effort will not only provide important theoretical insights into the process of speciation, but will also benefit the barcode community by providing biologically meaningful information for fine-tuning genetic thresholds used to flag provisional species.

DNA BARCODES COULD HELP TO IDENTIFY AND CONSERVE MEXICAN CACTACEAE

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DNA barcodes could be a useful tool for plant conservation. Of particular importance is the ability to identify unknown plant material, such as from customs seizures of threatened and protected species. Mexican cacti are an example of a threatened group since they are under pressure from wild collection for the xeriscaping trade and private collectors. Mexican cacti also provide a taxonomically and geographically coherent group with which to test DNA barcodes. Here we sample the *matK* barcode for 529 species of Cactaceae including approximately 75% of Mexican species, and test the utility of the *matK* region for species level identification. We find that the *matK* DNA barcode can be used to identify uniquely 78% of all species, and 79-84% of species of particular conservation importance. However, this is far below the desired rate of 95% and there are still significant issues for PCR amplification due to the variability of primer sites. Additionally, we test the chloroplast *rpoC1* and nuclear *ITS* regions for the cactus subfamily Opuntioideae and for the genus *Ariocarpus* (subfamily Cactoideae). *rpoC1* shows a high PCR success, but only 38% of Opuntioid species sampled have unique sequence. For *Ariocarpus*, six of the seven species have a unique sequence. In contrast, we observed much higher rates of variation for *ITS* (84% unique for Opuntioideae sampled) but a much lower PCR success, encountering significant intra-individual polymorphism in *Ariocarpus* precluding the use of this marker in this taxon.

PROJECT BARKCODE: ENGAGING SCHOOLS IN TREE-BOL

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A key justification for DNA barcoding is its potential to increase and improve public engagement with the natural world. CBOL's mission is not only to assemble barcode reference libraries, develop technology and encourage global participation of taxonomists, but also to promote 'the use of DNA barcoding for the benefit of science and society'. The Natural History Museum, London has partnered with a UK educational charity to explore how schoolchildren may be engaged now in the generation – not just use – of DNA barcode reference databases. 'Project BarkCode' will DNA-barcode the British tree flora while piloting methods for involving children and other non-

experts in DNA barcoding campaigns. From 2010 through 2012, over one thousand pupils from six Cothill Educational Trust schools and selected London schools will, under the guidance of NHM scientists at a purpose-built science education centre ideally situated in the Jurassic Coast World Heritage Site, prepare voucher specimens and sequence *matK* and *rbcL* from 2-3 trees each. Research objectives include the production of *matK* and *rbcL* sequences for up to 10,000 vouchered British tree and shrub specimens as a contribution to the first major plant barcoding campaign, Tree-BoL. Such intensive sampling of a small, tractable tree flora could serve as a test-bed for the effects of sampling density on DNA barcode performance in temperate floras where the overall number of species is both known and low but where frequent hybridization can lead to difficulties in species circumscription and specimen identification. Educational objectives include student participation in 'real science' rather than repeating canned experiments with known outcomes. Students will witness the significance of their own small but novel contribution towards current international research and conservation objectives.

THE PSBA-TRNH INTERGENIC SPACER REGION DATABASE-A WEB SERVICE FOR DNA BARCODING

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The psbA-trnH intergenic region (ptigs) is among the most variable regions in the angiosperm chloroplast genome. It is a popular tool for plant population genetics and species level phylogenetics and has been proposed as suitable for DNA barcoding studies. The first step in doing this is the correct determination and identification of the different regions in the PsbA-TrnH regions. Our initial analyses suggest that 104 out of 986 (10%) psbaA-trnH regions belong to the species of the family Orchidaceae are incorrect. This led us to use additional methods to correctly determine and isolate the psbA-trnH region. To do this, we first collected sets of training psbA-trnH sequences from the public databases. The flanking psbA and trnH regions were retrieved based on the annotations in the database. The regions were subjected to multiple sequence alignment and inspected manually. The resulted alignment was used to build hidden markov models (HMM). Test sequences were searched with these models and the various ptgis regions were determined based on the mapping of the HMM models on the sequences. We have tested our models on the families of Orchidaceae and Fabaceae. The curated psbA-trnH regions were then stored into a database, a web interface will be implemented so that the users can determine the structures of newly sequenced psbA-trnH regions and also to retrieve the curated psbA-trnH sequences directly.

ArBOL: A DNA BARCODING INITIATIVE FOR NEOTROPICAL PLANTS

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The neotropics are a biodiversity rich region with the highest per country species diversity indices; Colombia, Brazil and Ecuador topping the list. This has been the result of complex evolutionary processes which coupled with a dynamic geological history, together have assembled a wide variety of habitats such as the caatingas, cerrado, campo rupestre and mata atlantica of Brazil, the Andes with its cloud forests, paramos, jalcas and punas, the choco, and the amazon rain forest to name only a few.

ArBOL, is a consortium of Latin-American institutions and rainforest initiatives created to foster the creation of a catalogue of neotropical plant barcodes. During its first meeting in Bogotá in 2008, a team of 20 botanists and plant molecular biologists, representing 6 countries and 6 regional forestry initiatives discussed issues such as regional capacity, sampling schemes and sources. See http://ArBOL.uniandes.edu.co.

Several plant DNA barcoding projects in the region are underway, and through ArBOL we are promoting and coordinating barcoding efforts in the region.

CONVENTIONAL AND NOVEL DNA BARCODES FOR APOCYANACEAE

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DNA barcoding is useful for species identification and in exploration of biodiversity. In plants, different groups have concentrated on various chloroplast genes like *matK, rbcL, atpB, rpoC1* etc., as a potential DNA barcode. Apocynaceae has been chosen in this study, due to its economical importance. It includes 415 genera with around 4650 species. In this study, *matK, rpoC1* and *GermacreneD* genes were studied using both *in silico* and wet lab approach.

The available sequences for *matK* (77 species from 7 genera) and *rpoC1* (69 species from 5 genera) from GenBank were analyzed. The percentage variation is 3.6, 3.34, 10.8 and 11.8 respectively for *atpB, rbcL, rpoC1* and *matK* at genus level. These results suggest that *matK* and *rpoC1* are potential DNA barcode candidates.

Twenty species from 9 genera were collected from the Western Ghats, India. Partial gene products were amplified using universal primers and the amplicon sizes are *matK*- 860, *rpoC1*- 480 and *GermacreneD*- 1274 bp's.

The results are discussed for *matK* and *rpoC1*, as the *GermacreneD* work is in progress. Intergeneric and intrageneric variations were studied. The overall genetic distance for *matK* is 0.01, as calculated by Kimura 2 formula. Pairwise alignment within the genera showed the variation of Carissa- 0.89%, Holarrhena-1.03%, Rauvolfia- 1.5%, Plumeria- 1.05% to 1.9%, Tevetia-1.2% to 2.8%, Wrightia- 1.0%. Only 5 sequences are available for *rpoC1* and hence it is difficult to analyze at intrageneric level, but it show 3.34% variation at intergeneric level. The genetic distance is 0.026. No difference found between, *Asclepias tuberosa* and *incarnata*.

Exact barcode regions were identified for *matK* and it lies between 90-210, 249-310, 330-462, 610-685 and 710-730 bp's. For *rpoC1*, it lies between 290-355 and 480-510 bp's. Now, we are studying the novel *GermacreneD* to check its effectiveness as barcode candidate. From these analyses, we found that *matK* gene differentiates at the species level compared to *rpoC1*.

DNA BARCODES OF MEXICAN OAKS

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Oaks (*Quercus* spp.) are widespread in temperate and subtropical areas of the Northern Hemisphere and constitute important trees from the ecological and economic standpoints. In Mexico, about 150 species have been recognized but in most cases their identification has been a challenging task due to the extensive levels of leaf polymorphism caused by local adaptation and frequent hybridization.

The identification of oak species could potentially be facilitated by the development of DNA barcodes. In this work the uses and limitations of rbcL and matK, the plant barcode recommended by the CBOL Plant Working Group, were tested in 40 oak species that included members of the white (*Quercus*) and red oak (*Lobatae*) sections. Additionally, the discriminatory power of the noncoding region trnH-psbA was examined. Sequence analysis of rbcL and matK revealed zero to few nucleotide differences among the species examined. In contrast, sequences of trnH-psbA allowed the discrimination of the white and red oak sections; however, a limited number of nucleotide differences were found among oak species. The potential use of additional nuclear regions as DNA barcodes for oaks is also discussed.

COMPLIMENTARILY AND CHALLENGES OF DNA BAR CODES FOR TAXANOMIC DETERMINATION OF USEFUL PLANTS

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DNA Barcode enables the identification of plant to species level based upon plastid DNA. Sequencing of a standard region of DNA in plants functions for many wild plants using immature leaf tissue. The application of DNA Barcoding to useful plants such as those employed as food, medicine, raw material, and ornamental is more challenging than that for wild plants because of the evolutionary consequences of plant-human interactions (such as divergent selection of distinct populations of the same species, interspecific hybridization, ploidy and domestication). Anthropogenic plants have taxonomic categories (chemovars, cultivars, etc.) that chemotaxonomic and morphometric methods have not fully resolved; barcoding of DNA in plastids of maternal inheritance may compliment these approaches to determination of inter- and infraspecific taxa. Barcoding standardization of procedures permits the application of molecular techniques among various laboratories. DNA barcoding is new compliment that can balance the limitations of chemical, microscopic and macroscopic techniques. However, its application to useful plants is challenged by the low quality of DNA and by amplification of products that are smaller than 600 base pairs. This situation is based upon the processing of plant material prior to analysis, the use of mature tissue with high concentrations of secondary compounds among other factors. The DNA data bases of reference collections for authentication useful plant species are limited and do not represent the wide range of vegetal products from human management and selection. The results of DNA barcoding permit the identification of plants by "matching" known sequences or by estimating phylogenetic proximity. Hence such identifications can be made of: 1) multiple species in mixtures, 2) material lacking diagnostic characters (e.g, immature plants, plant fragments, etc.), 3) substitutions, contaminants, adulterants, "spiking" non-biological compounds and toxic additions, 4) geographically and taxonomically distinct members of useful plant complexes, 5) restricted species (e.g., controlled species, endangered species, etc.), among others. The governmental and non-governmental receptors of such DNA barcode identifications will be able to apply the results to: 1) quality control and regulation of vegetal products for human consumption, 2) enforcement of national and international commercial and conservation regulations, 3) industrial development and plant improvement (at transnational and local levels) as well as 4) basic and applied research. Examples from the literature survey illustrate the potential application of DNA barcodes. Emphasis will be placed on medicinal plants and the representation of important vascular plant families (e.g., Asteraceae, Euphorbiaceae, Apiaceae Solanaceae) in molecular information data bases.

COMPLEMENTING DIFFERENT MACROFUNGI CAMPAIGNS: TAXONOMIC AND REGIONAL INVENTORY PLUS FUNCTIONAL GROUP BIODIVERSITY STUDIES

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The number of macrofungi species worldwide has been estimated recently to be between 65,000 and 110,000 (Mueller et al., 2007). Following these authors'estimation criteria, the probable macrofungi species number in Mexico and Mesoamerica will be reviewed and compared to those of Cifuentes (1996) and Guzman (1998). A large difference is expected between described and non-described species.

Species identification in this group often is difficult due to the lack of reliable monographic taxonomic treatments, and the use of only the morphological species concept. Examples of estimated endemism areas with different species concept will be presented. The impact of DNA barcode data from the standard fungal nuclear ribosomal ITS region will be discussed.

Our efforts will focus primarily on a campaign with a taxonomic approach based on previous natural history collections of mushrooms, and well curated and monographed selected groups of aphyllophoroid, agaricoid and gateroid fungi. The perspectives of complementing different campaign approaches will be discussed.

CRUSTACEAN DECAPODS OF THE GULF OF MEXICO, SUPERFAMILY PENAEOIDEA

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Shrimp of the Superfamily Penaeoidea are keystone species in the marine ecosystem of the Gulf of México. They constitute a diverse group distributed from shallow to deep waters and represent an important component of food web. Some of this species are subjected to extensive fisheries exploitation and others are of potential commercial utilization. During the last 25 years. We have conducted intense sampling in the Southwestern Gulf of Mexico in depths ranging from 12 to 1200 m. A big collection of crustacean decapods has been gathered. Part of this biological material was preserved in alcohol. Penaeoid shrimp collection is composed by five families, 14 genera and 20 species. Previous molecular studies based on DNA mitochondrial sequences of penaeoid shrimps were analyzed to reconstruct the phylogeny of the Superfamily Penaeoidea. DNA barcoding of penaeoid shrimp will be developed as part of a larger project to characterize Decapod Crustaceans of the Gulf of Mexico.

MEXBOL, THE MEXICAN COMMITMENT TO DNA BARCODES

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Mexico is mega-diversity region from the world, every year no less than 100 species new for science from all living forms are described. Just to give an example, in case of fish fauna this country hosts almost the same number of marine and freshwater species than the USA and Canada together. Barcodes are a promising technique to describe and uncover hidden biodiversity, but the challenge is complex. In our country, in order to fulfill the goal to barcode of the most plants and animals, it has been created the Mexican Barcode of Life organization (MEXBOL), linked directly to the International Barcode of Life project. Additionally, MEXBOL forms part of a network supported directly by the Mexican federal ministry for science (Consejo Nacional de Ciencia y Tecnología, CONACYT). MEXBOL was just activated in March this year and received the fist part of funds (138,500 dlls) from a total $770,000 promised for this year. As well, additional funds to support barcoding and a mirror site for Barcode of Life Database (BOLD) will be provided by Comisión Nacional para el Uso y Conocimiento de la Biodiversidad (CONABIO). Our strategy was to generate three middle volume labs (about 15,000 reactions/year/each) to extract DNA, amplification of the gene COX I by PCR and get sequences. Those labs are distributed in the south, center and north of the country in three leading institutions: two research centers from CONACYT, El Colegio de la Frontera Sur (ECOSUR) and Centro de Investigaciones Biológicas del Noreste (CIBNOR), and the National Autononomous University of Mexico through the Instituto de Biología.

Actually, CONABIO has activated and financed with an amount around of 570,000 dlls. 20 projects on DNA barcoding for 9 institutions, after a careful peer review.

Despite the short time working, Mexican researchers have published results in DNA barcoding with 100 species in freshwater zooplankton and 61 freshwater fish. Three invertebrates highlighted by the barcodes, have been described as new for science after careful morphological and ecological analyses. We expect, once the labs and projects will be working at full capacity, to get additional support to Mexican Biorepositories, and to speed the process on the inventory of the biological richness, not only for Mexico, but to increase the exchange and support to Mesoamerican researchers.

DEVELOPMENT OF THE ACADEMIC NODE OF MEXBOL AT THE INSTITUTE OF BIOLOGY OF UNAM

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The Institute of Biology at UNAM became part of the Consortium for the Barcode of Life in 2007, and participated in the launching of the iBOL project in the same year. We hold national collections of biodiversity in numerous groups, such as the national herbarium, ten zoological collections, a botanical garden, and we participate in the management of three natural protected areas. We expect to contribute greatly to the Barcode of Life in Mexico. As a node in MexBol we intent to provide support not only to our researchers and students, but to our colleagues in many other universities or research centers that are interested in using our support, or would like to work in collaboration. As such, we are improving our molelucar lab capabilities. In this work we will present strategies developed in our Barcode of Life laboratories to become a mid-output laboratory in the Barcode of Life project. We will also summarize the people and groups they have started to process samples with us. At the moment of this abstract we are supporting at different levels 8 projects in different biological groups that have external funding, and 5 other internal projects that will search for additional funding in the future.

ALL TAXA BARCODING INITIATIVE IN CHAMELA-CUIXMALA, MÉXICO

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México is considered a megadiverse country. It is necessary to adopt strategies that facilitate the completion of an inventory of the Mexican biota and allow the planning of the management and protection of our biodiversity. In particular, the tropical dry forest is a system that occupies 60% of the area of tropical forests in Mexico, and hosts one third of the vertebrate diversity and endemism in the country, and 40% of the endemic species of vascular plants. In spite of its obvious importance for the national biodiversity, this ecosystem suffers one of the highest rates of deforestation. Chamela-Cuixmala Reserve is located in Jalisco state in north western Mexico. Its extention is 13,142 ha and is one of the better studied areas of tropical dry forest of the world. It represents an ideal model to undertake the All Taxa Barcoding Initiative of the species of the tropical dry forest in Mexico. Detailed knowledge about the morphological diversity of many taxonomic groups in the reserve is available, specially vascular plants, vertebrates and several groups of insects. This knowledge and the participation in the project of those specialists who generated it, ensures its success. Taxonomic groups initially included in the project are fungi, vascular plants, vertebrates, insects (Coleoptera, Hymenoptera, Odonata), crustaceans, annellids, acanthocephalans, nematodes and platyhelminthes. Intense collecting activities will be held in the reserve through collaboration of specialists in different taxonomic groups. Specimens from herbarium and collections will also be sampled and photographed when possible. At least 5

specimens per species will be sampled and pictures of the specimens will be taken. Around 1,000 species will be collected for an estimate of 8,000 barcodes in three years at least.

BARCODING A VERY COMPLEX TROPICAL TROPHIC WEB

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We have integrated DNA barcoding into the ongoing total inventory of the Lepidoptera (moths and butterflies minus leaf miners), caterpillar parasitoids, and caterpillar food plants, in the 120,000 ha terrestrial portion of Area de Conservacion Guanacaste (ACG). This large conserved wildland in northwestern Costa Rica is dry forest, rain forest and cloud forest, and a multitude of intergrades. The ACG biodiversity inventory began in 1978 and barcoding began in 2003.   5,000+ species have been barcoded among more than 100,000 samples.   All collateral data is databased and available through the project web site (http://janzen.sas.upenn.edu).

Among these insects, less than 0.3% of the species have such similar barcodes that they cannot be molecularily identified. Similar results are being obtained with the newly initiated barcoding of their food plants.   In addition to barcodes being an extremely reliable species identifier, the act of barcoding a large and species-rich array of morphologically identified species exposes an additional 5-10% Lepidoptera species and 20-50% additional species of parasitoid wasps and flies. These species are being confirmed as biological entities through differences in inconspicuous morphological traits, behavioral traits, microgeographic location, and caterpillar food plants.

This taxonomic exploration and confirmation of a large complex tropical fauna through barcoding is showing that while some "generalist" species are indeed that, most are complexes of similar specialist species. This is turn generates not only the need for massive amounts of alpha taxonomy, but also suggests that many so-called widespread species may turn out to be complexes of variously allopatric, parapatric or sympatric similar species.

In this presentation we use 500+ ACG species of the butterfly family Hesperiidae to illustrate these generalizations.

BIODIVERSITY OF GUATEMALA AND THE IMPORTANCE OF PARTICIPATING IN THE BARCODE OF LIFE

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The geographical location and highly variable topography of Guatemala favors its distinctive high biodiversity. A National Biodiversity Strategy has been developed to help prevent threats against biodiversity, and to promote its preservation. However, the lack of taxonomic studies makes the application of effective conservation strategies difficult. It is therefore necessary to conduct taxonomic studies that help in the identification of species, and this capacity would be increased with participation in the DNA Barcode of Life project.

The Natural History Museum of the University of San Carlos (USAC) has 40,000 specimens approximately and, in collaboration with the Museum of Vertebrate Zoology of the University of California, has established a collection of 1,500 tissue samples from vertebrates from mountainous regions of Guatemala, 25% of which has already been processed using Cytb.

In 2004, the Herbarium –BIGU- of the School of Biology initiated a macroscopic fungus collection, with 3,500 specimens approximately of at least 600 morfospecies, and intends to constitute a tissue collection to be used in species identification through molecular techniques.

Biological control also requires acute species identification. Laboratories of the USAC and other universities and private companies have collections of useful microbiological material that could be identified using molecular techniques.
The Laboratory of Entomology –LENAP- carried out bioassays and RAPD-PCR to determine if various strains of entomopathogenic fungus could act as biological control for *Triatoma dimidiata*, the Chagas disease' vector. In the aim to apply effective and ecological friendly control strategies of the disease, and using different molecular techniques (ITS, RAPD-PCR, ND4, Cytb) –LENAP- found out that the taxonomic identity of *T. dimidiata*, must be revised. Up to now, the bee team has already sent specimens of stingless bees to collaborate with the Bee Barcode of Life Initiative.

DNA BARCODE OF MIDAS CICHLIDAE SPECIES COMPLEX INHABITING LAKES AND LAGOONS OF NICARAGUA

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Midas Cichlidae Complex (species of the genus Amphilophus) has been the subject of debate among different groups of scientists since the first attempts at taxonomic classification. Problems were encountered due to the similarity in the morphology of some species. Initially it was thought that there was only one polymorphic species and, after conducting several studies, we know now that there are different species within the complex.

 We are using DNA Barcode as a molecular tool to identify the different cichlidae species that inhabit lakes and lagoons of Nicaragua. With this investigation we expect to contribute to the study of the Midas Cichlidae complex, and help resolve some issues related to it, such as the speciation mechanism involved and the true diversity of the complex.

BARCODING MEGADIVERSE *ENCARSIA* PARASITOIDS IN MEXICO

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The parasitoid wasp genus *Encarsia* is of major economic importance as a successful biocontrol agent of plant feeding insects. *Encarsia* is also a highly diverse genus in terms of numbers of actual and described species (400 described species and possibly 4000 actual species), genetic diversity (*Encarsia* shows greater variation in the 28S-D2 ribosomal gene fragment than is shown by the entire family Eulophidae), and biological diversity.

 Several species have been used very successfully as biological control agents, most frequently for invasive alien pest species. One such invasive alien, the spiralling whitefly *Lecanoideus floccissimus*, has recently invaded Tenerife from Central or South America. *Encarsia* species from Mexico are currently being collected and tested against this pest.

 Although the genus was recently revised in Mexico, the probable number of species present is much greater. We are using two gene fragments (CO1 and 28S) for barcoding the *Encarsia* species of Mexico, together with complimentary studies of morphology, including morphometrics. Our protocol is completely non-destructive, resulting in genomic DNA extraction and perfect, slide-mounted specimens, vouchers of which are deposited at UNAM.

Our studies highlight the built-in obsolescence of taxonomic revisionary monographs, and demonstrate the pressing need for online dynamic revisions that include critical molecular data. Web-based, dynamic taxonomies can be accommodated in the "scratchpads" developed from the EDIT project, while incorporating methods used by the Platygastroidea Planetary Biodiversity Inventory (PBI). While essential to the future of taxonomy, this approach will require changes to the current ICZN code that will permit electronic-only publication, coupled with registration in ZooBank.

FISH BOL STRATEGIES IN MESOAMERICA

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Mexico has the commitment to become a regional node in the International Project for the Barcode of Life (iBOL) thru MEXBOL network. This country could also be a natural link between other Mesoamerican countries. In case of fish, this region has a striking diversity, with endemic species from a sole lake in the central plateau of Mexico to the intermittent rivers in Guatemala, the crater lakes in Nicaragua, etc., many of them still unknown. For example, only in Mexico, in the last five years, at least 13 new species have been described, including one new genus of cichlids (*Rocio*) and a new family of catfishes, the Lacantuniidae. This region also hosts the second largest reef in the world running from north of Yucatan to Honduras and the biggest atoll in the world, Chinchorro Bank.

Several strategies have been developed to accomplish the challenge of barcoding all fish in this complex and diverse region. Firstly, ECOSUR, being in the south border of Mexico, has been intensively sampling this region and adjacent countries, in association with other researchers. In the central plateau, we have links with other institutions such as Instituto Politécnico Nacional and Universidad Nacional Autónoma de México (UNAM), and in the north with Universidad Autónoma de Nuevo León. Secondly, we organized, in association with the Consortium for the Barcode of Life Comisión Nacional para el uso y Conocimiento de la Biodiversidad (CONABIO), Consejo Nacional de Ciencia y Tecnología (CONACYT), and the Quintana Roo State Council for Science, the first meeting on North and Central American Linkages for the DNA Barcoding of Fish in El Colegio de la Frontera Sur (ECOSUR) at Chetumal City. Our actual and future plans are to continue spreading information about barcoding, organizing regional Fish-Bol meetings and making alliances to find ways to obtain funding. In addition we would like to introduce the Consortium for the Barcode of Life (CBOL), FishBOL, and Mexican Barcode of Life (MEXBOL) initiatives to the scientific communities of this region, and to show the results of our fish barcoding projects.

Poster Abstracts

Algae, Fungi, Protists & New Groups

DNA BARCODES FOR THE DISCOVERY AND IDENTIFICATION OF NEW SPECIES OF *PYTHIUM*

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DNA barcoding is a rapid approach for screening large collections and identifying potential new species. Many strains have lost the capability to readily produce taxonomically relevant morphological structures. DNA barcoding overcomes such limitations and can be used as a screening tool for biodiversity discovery. Approximately 1000 DAOM and CBS *Pythium* strains were analyzed based on their nuclear (ITS) and mitochondrial (COI) barcodes by neighbour-joining (NJ) methods to determine potential novel species. We already had multigene phylogenies of the entire genus *Pythium* using the ex-type of each known species. Morphological traits and phylogenetic analysis with ITS and COI using the species from the clade containing the new taxa were correlated and three new species among them were characterized and described as *Pythium oopapillum*, *Pythium emineosum* and *Pythium camurandrum* respectively. These three new species grouped in clades B, F and E, respectively. Maximum parsimony can underestimate evolutionary time and the variance in ancestral states. To resolve the uncertainty in the phylogeny and ascertain the robustness of the new species, we used maximum likelihood with a general time reversible (GTR) model of nucleotide substitution and estimated the probabilities of each possible state. Furthermore, Bayesian inference analysis with Markov chain Monte Carlo (MCMC) methodology was done to calculate the posterior probabilities of phylogenies and determine the accuracy in the occurrence of these new species in their respective clades. Our analyses suggest the following findings. (1) Maximum parsimony: In all the new species clusters, the consistency and retention indices were higher in ITS than COI. These were supported with higher bootstrap values. (2) Maximum likelihood: Log-likelihood ratios were found to be similar in *P. oopapillum* and *P. camurandrum* groups in both barcodes. Log-likelihood ratio was lower in COI than ITS in the *P. emineosum* group. (3) Bayesian inferences: Similar posterior probability values in both the barcodes in the *P. oopapillum* group. However, in *P. emineosum* and *P. camurandrum* groups, COI has slightly lower probability values than ITS. In conclusion, the phylogenetic position and ancestral states of these new species within their clades is consistent and is strongly supported by statistical analyses of the ITS and COI barcodes and is well correlated with phylogenies using other genes.

Poster Abstracts

Algae, Fungi, Protists & New Groups

BARCODES OF SCOLOPENDROMORPHA CENTIPEDES OF TAIWAN

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The traditional systematics of Scolopendromorpha centipedes is based on morphological characters. However, it is not easy to identify some species with morphological characters. In addition, factors such as the sexual dimorphism and morphological changes of the post larval development in some species, variations in the regenerations of legs and antennae, all adding difficulty and leading to errors in the identification and classification of Scolopendromorpha species. In the present study, we determined 44 COI barcodes for 14 species of Taiwanese scolopendromorphs, and analyzed their phylogenetic relationship with 23 partial COI sequences of Chilopoda centipedes from GenBank. The result indicates that the interspecies sequence identities and the intraspecies sequence identities vary with species. Both high minimum intraspecies identity and maximum interspecies identity are present in Genus *Scolopocryptops*. The phylogenetic trees divided Scolopendromorpha into three families, supported that a close relationship exists between Scolopocryptopidae and Scolopendridae, and confirmed that *Scolopendra multidens* should be a valid species, not a subspecies of *S. subspinipes*. Although the phylogenetic trees didn't distinguish among the genera *Rhysida*, *Otostigmus* and *Ethmostigmus*, the maximum parsimony bootstrap trees and the low maximum interspecies identifies showed that the COI barcoding method is one of good tools for identifying the species of Scolopendromorpha. The result indicates that the minimum intraspecies identity of *Rhysida immarginata* are very high (0.993), and two of five sequences from Lieyu Islet which is near China mainland, the other three from Taiwan Island. However, *R. immarginata* was never recorded in China mainland.

PRELIMINARY RESULTS ON OSTRACOD BARCODES FROM YUCATAN PENINSULA

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Ostracods from Yucatan have not been studied since 1936, when it was published a report from a survey from Carnegie Institution (USA). After this report, a list of 23 species, thirteen of them new to science was given. Moreover, four species were common with Africa representatives, and one of them (Darwinula stevensoni) was considered cosmopolitan. In our study of 50 water bodies from Yucatan, comprising the unique sinkholes (locally named cenotes), lakes and temporary pools, we identified 22 morphotypes, only nine of them could be identified to species level with the literature available. Seven morphotypes were coincident with the barcodes, and only three of them could be identified to species level with no doubt. A preliminary comparison with ostracods barcoded from Manitoba, Canada allowed to establish that material from Yucatan is entirely different suggesting a restricted distribution in the Americas for the species in this group.

Poster Abstracts

Algae, Fungi, Protists & New Groups

Nowadays, we are working to get more barcodes from our material but the preliminary results suggests a strong relationship between morphotypes and barcodes, as well as the possibility for the presence of some endemics in some unique systems from Yucatan, as it has been found in other groups of crustaceans and fishes.

DNA BARCODING FOR PERUVIAN *MEGALOBULIMUS* SPP. (MOLLUSCA; GASTROPODA)

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The genus *Megalobulimus* (Family Megalobulimidae) is a group of land snail widely distributed in the tropical forest areas of South America. It has been a source of food for people since ancient times. Nowadays, it has acquired economic importance due to its cosmetic and nutritional properties and it could be a promising taxon in the Peruvian biotrade. Getting DNA barcodes of the species would help in these purposes, as well as in promoting their sustainable use. The taxonomy in this group is not clear, a problem that DNA barcodes could help solve too. We surveyed the two most traded *Megalobulimus* species (*M. capillaceus* and *M. huascari*) from San Martin, a region in Peru with high preference of this meat. After total DNA extraction by CTAB method from foot muscle tissue, we amplified and sequenced a 706-bp fragment of the mitochondrial Cytochrome C oxidase subunit I (COI) gene (both strains) using COI universal primers (Folmer et al., 1994). Aligned sequences were analyzed by the following phylogenetic methods: Neighbor Joining, Maximum Parsimony, Maximum Likelihood and Bayesian Inference and also were evaluated genetic distances. We obtained 26 sequences of both species. Surprisingly, we only found one haplotype, from 17 individuals, for *M. capillaceus*. *M. huascari* has a difficult taxonomy due to its shell shape variation; however, the intraspecific genetic distance was 1.9%. The genetic distance between the two species was 19.53%. In conclusion, COI gene is a good marker to discriminate between those species and should be used as a DNA barcode for *M. capillaceus* and *M. huascari*.

DNA BARCODING OF PROTISTS IN CULTURE COLLECTIONS

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(12) Culture Collection of Autotrophic Organisms, Czech Republic
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The standard animal DNA barcode is a short sequence from mitochondrial gene cytochrome c oxidase 1 (CO1). A two-locus barcode from chloroplast genome has recently been proposed for plant barcoding. Fungal species identification has relied on ribosomal markers and a barcode system based primarily on ITS sequence have been discussed. Protists encompass an incredible diversity and hence have been a challenge for DNA barcoding. In addition, their small size and cryptic morphology make it difficult to develop standard high throughput tools for their barcode analysis. Here we report on an effort on developing a high throughput protist barcoding system utilizing 570 DNA samples from eight international protist culture collections. The samples we tested cover a large taxonomic range from Stramenophiles, Viridiplantae, Chlorophyta; Glaucocystophyceae and Rhodophyta. We attempted sequencing four loci including mitochondrial CO1 barcode region and three nuclear ribosomal markers LSU, ITS and SSU (as traditional loci used in protist biosystematics for almost 20 years). Different PCR strategies were tested for each marker to obtain optimal amplification. CO1 amplification was variable depending of the taxonomic group and on average we obtained amplicons in 47% of the samples. Ribosomal LSU amplified successfully in 66% of the samples while ITS and SSU produced significantly more variable results depending on taxonomic group and primer sets tested. Based on these results CO1 and LSU provide better possibilities for developing a high throughput barcoding system for protists. However, because CO1 is a protein coding gene with relatively conserved amino acid structure, it is easier to align as compared to ribosomal LSU. Although we note that species-level resolution may be difficult to achieve using any single gene and that protist barcoding will likely require a multi marker system.

Poster Abstracts

Algae, Fungi, Protists & New Groups

DNA BARCODING REVEALS NEW FRESHWATER ZOOPLANKTON SPECIES

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DNA barcoding was used as a complementary tool to analyze the diversity and distribution of freshwater zooplankton in Mexico. Several species new to science as well as new records for Mexico were revealed by the COI sequence data. Two species were already described: the cladoceran *Leberis chihuahuensis* from the northern semi-desert region and *Leptodiaptomus garciai*, a microendemic copepod from an ancient saline crater lake in central Mexico. This last species was previously synonymized with *L. novamexicanus*. Here we describe two more cryptic species highlighted by DNA barcoding. The first species is a cladoceran of the genus *Scapholeberis* (Cladocera: Anomopoda: Daphniidae). This species was found in the northern semi-desert region of Mexico and displays morphological characters highly similar to those of *S. armata freyi*. With a mean divergence of 12.2% in the COI sequence between this species and *S. armata freyi*, we found consistent morphological differences between them. The second species is a copepod of the genus *Mastigodiaptomus* (Crustacea: Maxillopoda). This species, found in the southeast of Mexico, coexists with *M. reidae* in a small permanent pond. A mean COI divergence of 19% and consistent morphological differences reveal that the new copepod is different from *M. reidae*. Experimental and field research is under progress to analyze reproduction and time-coexistence of these two *Mastigodiaptomus* species.

DNA BARCODING OF VISVA-BHARATI CULTURE COLLECTION OF ALGAE

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Microalgae are a highly diverse group of unicellular organisms comprising the eukaryotic protists and the prokaryotic cyanobacteria or blue-green algae. The microalgae have a unique environmental status; being virtually ubiquitous in euphotic aquatic niches, they can occupy extreme habitats ranging from tropical coral reefs to the polar regions, and contribute to half of the globe's photosynthetic activity. The Visva-Bharati Culture collection of algae (VBCCA) was established in Department of Botany and is affiliated to World Federation for Culture Collections (WFCC) and having accession no WDCM931. The isolates are from fresh water as well marine habitats, soil crust, rice field soils and biofilm from subaerial habitats and presently holding 240 species/strains. Many of the strains having similar in morphology and often confusion in identification. Some of the species even when collected from nature are different in morphology but while in culture having similar morphology. We are interested in applying DNA barcoding to algal strains of our culture collection. Though cytochrome c oxidase 1 has been shown to be a useful tool for differentiating some groups of marine macro algae, its wide application in the micro

algae has yet to be studied. We have just started the project and wish to collaborate with the International network like Canadian Barcode of Life Network to complete successfully the project. During the conference we will present about our culture collection and our plan for DNA barcoding of strains of our culture collection.

MOLECULAR TAXONOMY OF DENDROBAENA BYBLICA SPECIES COMPLEX IN IRAN

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Phylogeny of Dendrobaena byblica species (Rosa1893) complex was reconstructed based on sequences of mitochondrial cytochrome C oxidase, subunit I (CO1). Seventy adult earthworms were collected from forests of Amol, a city in northern Iran. They were classified into two OTUs based on morphology of their prostumiom and body color, consistent with previous groupings of this species complex. Samples for DNA extraction and subsequent PCR and sequencing were taken from caudal tissue to prevent contamination by gut contents. Initial sequencing of a ~700 bp CO1 fragment in two samples of each group suggested one of the groups can be further subdivided. Ultimately, CO1 was sequenced in 15 individuals. The sequences were analyzed using MEGA4 software, and the results clearly grouped the organisms into three clades. The genetic divergence and morphological variations among these OTUs suggest that this species complex may consist of at least three semi-species. The anterior part of each earthworm has been kept in 90% ethanol, and detailed morphologic analysis is being done to detect possible concordance with the molecular data.

Poster Abstracts

All Bird Barcoding Initiative & Vertebrates

DNA BARCODING OF EUROPEAN ACCIPITER AND THEIR AFRICAN RELATIVES

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We used museum specimens of African and European Accipiters for a DNA based analysis of the morphologically established species and subspecies boundaries (Handbook of the Birds of the World 1994). In general, DNA-barcoding confirms the morphologically recognized bird species, but there are exceptions.

DNA BARCODING OF SOME AMPHIBIANS OF WESTERN GHATS

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The Western Ghats of India are one of the hot spots of amphibian diversity with 60% of the Indian amphibians of which 80% are endemic. However, taxonomic ambiguities are prevalent due to the lack of taxonomic description/uncertainties/morphological similarities or the loss of type specimens of many known species. As a first step to resolve this, the present work designed to differentiate 20 amphibian species of Western Ghats using mt DNA markers. Protocols followed in the present work include DNA isolation by non-invasive methods, PCR amplification using universal primers and sequencing of four mitochondrial genes such as COI, Cyt b, 16S and 12S rRNA. Partial sequences were aligned and constructed trees by using Bioedit and MEGA softwares. This forms the first report of DNA barcoding sequences of amphibians of Western Ghats. Comparative performances of these four markers for species resolution were described and discussed.

PRACTICAL APPLICATIONS BEYOND TAXONOMY: GENETIC TOOLS TO MONITOR CAPTIVE DOLPHINS IN AQUATIC PARKS AND DELPHINARIA

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Live small whales and dolphins are a highly significant trade product nowadays for touristic aquatic parks and delphinaria. Controversy has arisen around the exportation of dolphins, particularly bottlenose dolphins (Tursiops truncatus), from sources that may have been overexploited or to which the carrying capacity to export animals in a sustainable way has not been assessed. It is also unknown to what extent individual dolphins that are captured and trained for aquatic parks die during collection, transport or after life in captivity, and whether they are exchanged for new captured animals without responsible record keeping. Some facilities use Passive Integrated transponders (PIT) tags to identify individuals, but this technique is not 100% accurate as they can malfunction, migrate through the skin, or even be removed

We propose that, as barcoding uses a standardizing technique to unambiguously identify species, the development of a similar technique to identify individuals is needed. General genetic markers that could aid the recognition of individual captive dolphins are crucial for two main purposes: to confirm the source population from where they were extracted and to monitor their movement and welfare in captivity. We present preliminary data of over 100 samples of bottlenose dolphins from captive and wild populations of the Gulf of Mexico and the Caribbean. Individuals were genotyped with 17 nuclear microsatellites that are widely used in bottlenose dolphin population structure studies worldwide. Assignment tests were carried out for each individual to confirm their population of origin. The use of microsatellite fingerprinting serve as a first approach to confirm individual captive dolphin's origin, as well as an individual identifier that could be used in the management of this protected species.

Poster Abstracts

Barcoding Species for Quarantine/ Plant Protection

DNA BARCODING OF AGROMYZID LEAF MINERS AND THEIR PARASITOIDS IN BANGLADESH

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A USDA funded 3 year long research project on Molecular Taxonomy and DNA barcoding of Agromyzid leaf miner pests of agricultural crops and their parasitoids in Bangladesh has been initiated. The Inception Seminar of the project was held at the Department of Zoology, University of Chittagong which was attended by teachers and post graduate research students from various departments of the Faculty of Biological Sciences. The research will reveal the intensity of pest activities in different parts of Bangladesh. Lists of crops affected by leaf miners, agromyzid pest insects and their parasitoids will be prepared. USDA scientists from Systematic Entomology Laboratory of the NMNH and MSC ARS will be coming to Chittagong for conducting Training Courses on Insect Taxonomy and Molecular techniques related to DNA barcoding. About 15 young teachers and post graduate students from Zoology, Botany, Microbiology, Biochemistry, Marine Sciences and Forestry will be recruited to be trained on DNA Barcoding. At the end of the project a DNA Barcode library will be established at the Insect Museum of the Department of Zoology of the University of Chittagong. A field guide book for the use of researchers and farmers will also be produced.

COMPREHENSIVE BARCODING OF AUSTRALIAN HELIOTHINE MOTHS (LEPIDOPTERA: NOCTUIDAE)

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Heliothinae (Lepidoptera: Noctuidae) is a cosmopolitan subfamily of some 365 species, including some of the world's most injurious crop pests (e.g. *Helicoverpa* and *Heliothis* spp.), regarded as serious biosecurity threats. Several previous studies have developed molecular diagnostics to distinguish a few members of this group, but none have examined more than four species simultaneously. We DNA barcoded the entire Australian heliothine fauna of 38 species, using the same museum specimens used to revise the Australian fauna last decade (Matthews 1999). Specimen ages ranged from 10-44 years, and most had been 'relaxed' and spread, resulting in DNA degradation. Obtaining barcode data was technically challenging but feasible. We included exotic species in our data set in order to test the utility of barcodes for quarantine diagnostics.

Within genera, uncorrected distances among species ranged from 8 % in *Adisura*, to 0 – 0.6 % among a terminal group of eight *Heliocheilus* species. The latter eight species cannot be distinguished using COI barcodes, but morphological differences in this group are subtle and even genitalic characters are not diagnostic (Matthews 1999). Despite incomplete resolution of the

barcode tree, all economically important species sampled are easily distinguished from each other and from non-economically important species. Additionally, all Australian native species, including *Heliocheilus* spp., can be distinguished from exotics. Thus DNA barcode data provides the important information required by quarantine authorities. Barcodes also identify two potential cryptic species within the recently revised Australian fauna.

Decades-old pinned insect specimens can be used routinely for DNA barcoding to fill sampling gaps although PCR success rates are perhaps halved and costs are doubled, using current technologies. The establishment of DNA barcode libraries for other taxa of quarantine significance will likely be equally profitable.

BARCODING TRUE BUG SPECIES OF INDIA

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Considering their pest status on a large variety of plants, the rapid and accurate identification of bug species is desirable for the implementation of control measures. Identification using conventional methods is time-consuming and requires an expert taxonomist. Also immature insects (larval stages) cannot be assigned to species. One of the aims of DNA barcoding is to focus on assembly of reference libraries of barcode sequences of known species. Surprisingly there are a very few true bug (Hemiptera) COI sequences deposited so far even though bugs are known to cause severe damage to plants and transmit viruses.

The present study reports COI sequencing of plant pest bugs. Our focus in this initial stage of barcoding activity is on three families namely Pentatomidae, Coreidae and Lygaeidae. Fifty COI sequences have been generated so far using universal primers described by Folmer et al. (1994) and barcodes have been obtained for twenty five different species. Besides adult specimen, nymphs were also used in some cases and COI sequences were compared with available database using BLAST. The unique sequences were deposited in GenBank. Our observation is that DNA barcoding can successfully assign the species even with nymph and damaged samples as starting material, which otherwise is not possible using conventional taxonomy. Phylogenetic tree constructed using available COI sequences clearly shows that DNA barcoding works well in congruence with conventional taxonomy. Additionally, we have obtained COI sequence for some interesting aquatic bugs including Giant Water Bug for which only few species have been described so far. All these studies mark the beginning of barcoding efforts and generation of database for future work on true bug species in India.

Poster Abstracts

Data Analysis Working Group (DAWG)

MORPHOLOGICAL CHARACTERISATION AND DNA BARCODING OF TWO CONGENERIC GONOMETA SPECIES IN MWINGI (KENYA

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Morphometric studies of males of two sympatric putative species of Gonometa, which have distinct morphological differences from Nuu, Nguni and Mituki in Mwingi district (Kenya) was done. Analyses of forewing measurements and body length measurements
using Principal Component Analysis(PCA) did not show any difference between the two morphologically different moths. DNA barcoding, which focuses on a 648-bp subunit of the mitochondria cytochrome oxidase 1 (5' COI) gene, which is a molecular tool, was used to compare the molecular variations between these two groups of morphologically different moths. The molecular results were matched against the morphometric results.

To get voucher specimens, moths were put in a freezer, followed by pinning them on a setting board. Mophometric measurements were taken on the specimen and analyzed using Statistical Analysis System software version 9.1.2. DNA was extracted from the middle left leg of the moths using the CTAB DNA extraction protocol. The COI region of the mitochondrial DNA was amplified using universal primers and direct sequencing was done on the cleaned PCR product. Analysis of the COI region was initially done using the Chromas software program to edit the sequences. Clustal X software program was then used to do multiple sequence alignments to check for any segregating sites within the sequences. Drawing a Neighbour-joining tree using Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 followed this to observe the distance between the two morphologically different wild silkmoths. Using this approach helped in discovering a new congeneric species of Gonometa in Mwingi that had not been described before.

THE MIRROR SITE OF BOLD SYSTEM IN CHINA

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The international Barcode of life project (iBOL) has organized an international collaboration aiming at the design, development and deployment of the use of DNA barcode to identify the living creatures around us. To accomplish this goal, we need both efforts from biology and that from informatics. Currently, the Barcode Of Life Data Systems (BOLD) has been constructed and are in use. As a central node of iBOL, China has been developing the mirror site of BOLD system. The BOLD mirror site is also a essential component of the information system of the barcode of life in China. The mirror site in China is designed to synchronize the data with other nodes periodically,

and provide the species identification and bioinformatics functions. The database and bioinformatics platform are designed loose coupled, and the connection are through web services and/or http services. Nevertheless, the mirror site in China is more than a duplication of BOLD. To meet the Chinese users' need, we are developing the Chinese tags for the data, and making translation between English and Chinese. We also placed some basic information of DNA barcode and some useful links (in Chinese) to let Chinese society to know DNA barcode better. Moreover, the mirror site is seamless to connect our information system, which contains the data submission system for Chinese collaborators and may provide further information of a given species and/or DNA barcode. Thus, our mirror site also functions as data transformer to transform the data from Chinese collaborators into BOLD system. Currently, we have integrated the international data from BOLD into our mirror site, that contains over 140,000 data entries. Those data can be accessed either by taxon search or by BLAST. We are developing the data transform rules to make the collections of fish, birds and fungi data in China into BOLD system. By now, the mirror site is still under construction.

PHYLOGENY OF MESOBUTHUS EUPEUS (C.L.Koch, 1839) IN IRAN USING CO1 SEQUENCES

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The genus Mesobuthus belonging to family Buthidae is most diverse in Central Asia and Iran. The scorpion M.eupeus is one of the most naturally widespread members of the family Buthidae, being present in eastern Turkey, Caucasia, southern Russia, Middle East, Central Asia, southern Mongolia and northern China. Additionally, considerable morphological variation has been attributed to populations of M.eupeus within this extensive geographic distribution and 21 recognized valid subspecies have been described. A recent comprehensive update or revision of M.eupeus is not available.

In this study, the first molecular phylogeny assessment of M.eupeus in Iran based on sequence data of a ~700bp fragment of Cytochrome C oxidase, subunit I is presented. Fifteen populations collected from different localities within Iran were included in the study. Phylogenetic relationships were inferred using neighbour-joining (p-distance) and maximum likelihood under GTR+Γ substitution model. The results support monophyly for M.eupeus, but they do show a clear deep split between northern and southern clades within the species. The northern clade included M.e.eupeus, M.e philippovitschi, M.e.afghanus, and M.e.thersites, while the southern clade comprised M.e.phillipsi and M.e.kirmanensis. In the topology observed, the "southern clade" has a basal position relative to the "northern clade". Accordingly, some scenarios for the evolution and phylogeographic structure of this species based on the paleogeography and geological history of Iranian plateau were proposed and tested. In addition to the intraspecific divergence, our topology showed a deeper phylogenetic split within the genus Mesobuthus. Here, a "western clade" consisting of M. gibbosus and M. cyprius is separated from an "eastern clade" containing M.

eupeus and M.caucasicus. Finally, the molecular clock hypothesis using the likelihood ratio test was applied in order to test the constancy of the evolutionary rates across the tree.

Poster Abstracts

Fish-BOL

A NEW SPECIES OF PARAPERCIS (TELEOSTEI: PINGUIPEDIDAE) IN WATERS OFF NORTHEASTERN TAIWAN BASED ON

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A new species of pinguipedid fish, Parapercis n. sp., differing from its sister species, Parapercis sexfasciata, is described in waters off northeastern Taiwan based on morphological and molecular data. In 30 specimens for each species we studied, the new species can be distinguished from P. sexfasciata by following distinct characters: the number of first pair sensory pores on the subopercle, several small black spots on the base of pectoral fin, the yellow line on the lateral side and no black spots on the base of dorsal fin. In addition, the 650 base pair of mitochondrial cytochrome I (COI) and 370 base pair of control region sequence data showed 7.5% and 16.3% of sequence divergence between these two species. The neighborjoing algorithm also revealed that two species was monophyletic. In conclusion, the two color morphotypes of P. sexfasciata could represent two different species which was clearly supported by the molecular and morphological data.

COMPARATIVE ANALYSIS OF TWO SPECIES OF PROFUNDULUS (PISCES: PROFUNDULIDAE), USING TWO GENETIC MARKER

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The fish Family Profundulidae endemic in the south-southeast Mexico, with five known species, at the specific analysis of the genus by means of morphological characters, is insufficient and complex due to the great fenotypic and intraspecific variation. The same that could even be an indicator of the existence of forms not described. The taxonomic study requires of the implementation of new tools. In this study the genetic composition is compared, in two species of the State of Chiapas by means of their molecular attributes, observed in different populations of the species P. candalarius and P. labialis. The biological material from 12 localities where both species live in simpatry, were identified until the species category. The DNA was isolated by means of the phenol/chloroform technique, and the cytochrome b and the region COI were amplified, using two different primers, products were direct-sequenced. The results are presented concluding that at least two of the populations of P. labialis can be considered as different.

Poster Abstracts

BARCODING FRESHWATER FISHES FROM COASTAL RIVERS OF SÃO PAULO STATE, BRAZIL

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The group of Southeast Atlantic drainages, called coastal rivers, comprises several watercourses that born in several coastal mountain chains and drain into the Atlantic Ocean. This region has suffered intense human activities, which have changed its natural conditions. About 48 fishes species are recognized for this basin. Although in numerical terms is taxonomically less diverse than others basin from Neotropical region, it is very rich in endemic forms produced by its long term independent evolutionary history. Additionally, several species of this area at extinction risk. Presently we analyzed about 50% of all species found in this basin. None of sequences showed insertions, deletions or stop codons. Almost all analyzed species were correctly identified. The average values of K2P distance are 2.9% for species, 12.3% for genus and 14.0% for families. The values presented are similar with that found in others DNA barcode fishes works. Thus, we conclude that DNA barcode is an efficient method for species-level identification of the neotropical freshwater fishes from coastal rivers basin from São Paulo state.

DNA BARCODING SOLVES THE TAXONOMIC AMBIGUITY PERSISTED WITHIN MUGILLIDAE

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An effort to justify the efficacy of 650 bp Cytochrome C oxidase subunit I (DNA barcode) gene in delineating the members of marine fin fishes belonging to taxonomically ambiguous family Mugilidae was attempted. In family Mugilidae morphometric conservatives resulted in ambiguous taxonomy. To address the issue we used all 95 barcode sequences of Mugilidae family available at NCBI (National Centre for Biotechnological Information) along with the barcode data generated from Mugilidae fishes of Parangipettai coastal waters for justification. The average GC content of Mugilidae was found to be 46.46%. Crenimugil crenilabis showed less GC content (44.55%) whereas Liza macrolepis holds high GC content (48.53%) among the Mugilidae members. The phylogenetic and genetic distance data relieved that Mugil platanus and Mugil liza represent the continuum of same species. Among the members of Mugilidae, the genus Mugil sp. might possibly contains more haplotype diversity relieved by intra-species genetic distance data. Species within genera of Mugilidae family invariably clustered in single clade. The clades revealed after bootstrapping generally corresponded well with expectations. We conclude that COI sequencing (barcoding) can be used to identify the individual members of family Mugilidae.

INHERITANCE STUDY USING ISSR TECHNIQUE, BETWEEN THREE MARINE FISH SPECIES OF SPARIDAE FAMILY FROM SYRIAN WATERS (EAST MEDITERRANEAN)

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The study was undertaken to check the relativity between Boops boops, Pagrus caeruleostictus and Pagellus acarne from the Sparidae Family by using ISSR technique because it is more advanced and reliable than RAPDs technique. ISSR needs more primers of nitrogenic bases, than using a higher and more specialized temperature and annealing, we used in this technique 21 primers. The products P CR were separated on acrylamide gel. We used the vertical electrophoresis device and ethidium bromide staining.

The results of the study were:
-   The study confirmed that the three kinds belong to the same family.
-   Boops boops and Pagellus acarneare more developed than Pagrus caeruleostictus.
-   Inconsistency rates between Boops boops and Pagrus caeruleostictus reached to 58%.
-   We noticed acceptable differences between the members of one kind for the three kinds, the least of them were in Pagrus caeruleostictus, whereas in Boops boops we noticed one member is greatly different than the oter two members in the same kind.
-   The Three kinds were put in two lineages: Boops boops and Pagellus acarne were in the first lineage, and Pagrus caeruleostictus was in the second lineage .
-   From the genealogical tree we noticed that the difference between Boops boops and Pagellus acarne was 52%

PHYLOGENETIC RELATIONSHIPS FOR SOUTHAMERICAN PINKLING GENUS *GENYPTERUS*

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Within the modern fish, Order Ophidiiformes out for its high diversity, comprising 5 families, been Ophidiidae the largest family with 46 genera, including *Genypterus*, for which phylogenetic hypotheses have not been evaluated, with unknown ancestry and descent relationships within the group. From 30 sequences obtained from the COI gene since the project FishBarcode for fishes from Australia, Argentina and new specimens from Chile, phylogenetic relationships among species of the genus *Genypterus* in South America are assessed. Bayesian approaches support an evolutionary history where *G. blacodes* is presented as the ancestral species, conforming *G. brasiliensis*, *G. chilensis* and *G. maculatus* an endemic South American clade (p-value = 0.80). The node root showed high posterior probability support (p-value = 1.0) suggesting a monophyletic status of the genus *Genypterus*.

MOLECULAR IDENTIFICATION OF FISH LARVAE OF THE BICOL SHELF, PHILIPPINES

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Morphological characters that are used in the identification of larvae of different fish species are difficult to distinguish and may overlap with other species. Thus, species identification of fish larvae can be hard and tedious. Advances in genetic methodologies have made it possible to identify larvae up to species level correctly. This study used DNA barcoding to determine the species composition of fish larvae collected from the Bicol Shelf. DNA extracted from the fish larvae samples were amplified with primers for the mitochondrial cytochrome oxidase I gene (COI) and sequenced. The samples consisted of larvae from 23 stations from two oceanographic surveys of the Bicol Shelf, in 2006 and 2007. The samples were initially morphologically classified and grouped into some commercially important taxa in the region namely: Carangid, Lutjanid, Scombrid, and Siganid.

The DNA sequences were matched with the Barcode of Life (BOLD) and Genbank databases, with many of the samples recognized to species level. Carangids include the shortfin (*Decapterus macrosoma*), Indian (*D. ruselli*), mackerel (*D. macrosoma*), bigeye (*Selar crumenophthalmus*), and torpedo (*Megalaspis cordylla*) scads; Lutjanids include the five-lined (*Lutjanus quinquelineatus*), dory (*L. fulviflamma*), Malabar blood (*L. malabricus*), black and white (*Macolor niger*), dirty ordure (*Paracaesio sordida*) snappers, and banded driftfish (*Psenes arafurensis*); Scombrid samples were the frigate tuna (*Auxis thazard*), skipjack tuna (*Katsuwonus pelamis*), and yellowfin tuna (*Thunnus albacares*); Siganids identified were *Siganus spinus*, *S. guttatus* and *S. fuscescens*. However, other samples returned with several species match or no species match at all. The present study represents an important first step in the use of DNA barcoding as a tool to confirm species identification of fish larvae and as a tool that could contribute to management and conservation studies of fisheries resources in the region.

DNA BARCODE: FISH SPECIES IDENTIFICATION IN THE IZS LABS

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Even if the EU confirms a sizeable consumption of seafood, according to ISMEA 2008 data the Mediterranean sea suffered a decline in its haul (-6.5%), with a resulting decline in national fish production (-3.1%).
Increased demand and contemporary difficulties of the seafood industry in meeting internal needs have increased dependency of EU from other countries to supply seafood products. Today imports

are making up for 70% of total resources: globalization explains the strange paradox between the reduction of stock and abundance of fish species in fish markets. Asian and African competitors are affecting the entire community system: is difficult to recognize foreign species morphologically similar to more valuable Mediterranean species.

Despite the morphological identification, innovative molecular biology techniques allow genetic recognition of "processed" samples as well: 'DNA Barcoding' uses 648pb region of cytochrome oxidase I (COI) as a "molecular key" to identify many taxa.

Through membership in the FishBol project and following the quality standards required, the Istituto Zooprofilattico Sperimentale (Genoa), the Museum of Natural History 'G.Doria', and the Minho University are cooperating in research funded by the Health Ministry.
Said project provides for the sequencing of the COI gene of about 50 fish species taken from the Mediterranean and European Atlantic. DNA barcodes, photos, and collection info of fishes examined will be included in a database, with the ultimate goal of DNA comparison of species from different areas, for the assessment of genomic differences.
Moreover, as the IZS is an official foodstuffs controlling entity, it may use a standardized and reproducible method for routine checking of seafood, to ensure the products traceability and to evaluate the transparency of information provided to consumers.
Finally, it may increase support to value the impact of "commercial fishing on fish stocks" in terms of biodiversity.

EVALUATION OF ENVIRONMENTAL POLLUTION AND ITS IMPACT ON FISHES IN CAUVERY RIVER, TAMILNADU, INDIA U

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Over the last decades aquatic ecosystems have been contaminated by persistent pollutant of Agriculture and industrial are in. The aquatic ecosystem has been affected by potential hazards including various environmental and industrial chemicals, heavy metals and biological agents which are discharged as a result of human activities. These exogenous materials impose abiotic stresses on aquatic organisms and affect entire biological process in terms of development physiology and event genetics. Currently aquatic animals have often been used in bioassays to monitor water quality. The development of biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentration of direct acting toxicants. Hence the proposed work is to study the genetic variation of the fishes among the different polluted water. The genetic monitoring through barcoding of these fishes can contribute information useful to protecting their health, conserving the long term viability and providing management options. The present deals with the sampling of fishes from six stations consisting of different polluted water (Sugar, steel, leather, chemical, and distilleries, dye). Genomic DNA were collected from the fishes and it was amplified by using PCR with universal primers so as to identify

the genetic variations. In addition Random Amplification of Polymorphic DNA PCR was done using primers of unknown sequences to elucidate genetic diversity. Further, basic water quality parameters (pH, TDS, BOD, COD etc) were carried out. To known the effect of toxicants enzyme activities such as AP, SGOT and SGPT were analyzed. Bioinformatic tools were also used in the present study to construct primers and to confirm genetic variation among the fishes. The results of the findings will be discussed in the presentation

BARCODING AND PHYLOGENY OF FLATFISH FROM NORTH-WEST PACIFIC AND SURROUNDING WATERS

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Flatfishes are commercially important group of fish species. They are widely distributed in the Pacific waters. Main focus of the research was on the family Pleuronectidae as most abundant in the North-West Pacific (NWP). This family is one of the largest in the order and widespread in the waters of the Russia Far East and on opposite coast of Pacific Ocean.The primary sequence of nucleotides at cytochrome oxidase c subunit 1 (Co-1) gene was determined in a frame of Fish-BOL in Russia. Original sequence length was in the limit 1500 bp. Taking into account the GenBank (NCBI) data, 22 species represented by 46 sequences of flounders and two out-group species of Gadiformes were analyzed at Co-1. For comparative purposes we used Cyt-b gene (1141 bp) that included 39 species both our and GenBank data.Phylogenetic relationships among representatives of flounders were based on four types of trees: neighbor joining, maximum parsimony, Bayesian and maximum likelihood. These trees showed similar topology, proving the monophyly of Pleuronectidae. Monophyly for this family has been suggested earlier basing on the results for 12S, 16S rRNA, Cyt-b and morphology. Two separate clusters on the trees, which support genus Pleuronectes subdivision and polyphyletic status of this taxon, were detected for Co-1 and Cyt-b genes used. However, according to last taxonomic revision (Cooper, Chapleau, 1998) we had only one species of the genus in our sample, Pleuronectes pinnifasciatus. Other species placed to Pseudopleuronectes, Liopsetta and Lepidopsetta. Individuals, which belong to the same species, were clustered jointly in a single node giving fine substantiation for species diagnostics (barcoding) based on Co-1. Barcoding on per individual basis was efficient both for the Cyt-b and for Co-1 genes.

Poster Abstracts

Insects & Terrestrial Arthropods

EXPLORING SHALLOW SPLITS IN COSTA RICAN BARCODED LEPIDOPTERA

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A total on-going inventory of 10,000 species of Lepidoptera of Area de Conservacion Guanacaste (ACG) in northwestern Costa Rica has integrated DNA barcoding to assist in identification and in discovery of new and cryptic species. Over 70,000 individuals from this inventory have been barcoded in this densely sampled DNA regional barcode campaign. Barcoding has revealed numerous cases where a morphologically-defined species contains two or more sympatric barcode clusters in an NJ tree. At least half of these (often shallow but consistent) cases are found to be supported by morphological and/or ecological differences, reinforcing their status as different (usually undescribed) species. However, a significant number of these cases lack these correlates and therefore could be cases where nuclear pseudogene copies of mitochondrial genes (NUMTs) have been amplified from some conspecific individuals, while the true barcode has been amplified from other conspecifics, giving the result of two adjacent clusters in an NJ tree. Most NUMTs show obvious signs of pseudogenes, such as stop codons and frame shift mutations, thereby allowing their rejection as barcodes. However, where this rejection is not possible, deeper genetic exploration is required to resolve the conundrum.

We are systematically examining cases of sympatric CO1 splits (that are not morphologically supported) within the ACG inventory, across many families, using different molecular tools. We are cloning and Sanger sequencing the 658bp CO1 sequence to detect clustering patterns of daughter sequences obtained from each clone. Our preliminary analysis of 72 clones for each of 4 species of ACG Sphingidae that have well-defined splits (Aellopos ceculus, Cocytius lucifer, Pachylia ficus, Xylophanes porcus) revealed no stop codons, frame shift mutations, or clustering of daughter sequences with their associated sister lineage. These results suggest that these shallow splits are not the result of one cluster being NUMT sequences. We are also comparing the cloning analysis with deep amplicon sequencing by means of next generation 454 sequencing. In a later phase of this project we will use nuclear markers to augment the results obtained by sequencing CO1.

Poster Abstracts

Insects & Terrestrial Arthropods

DNA BARCODING DEMONSTRATES NEED FOR TAXONOMIC REVISIONS IN THE MIDGE FAMILY CHIRONOMIDAE (DIPTERA)

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Chironomidae, non-biting midges, is the most diverse, abundant and widely distributed family of freshwater insects. Because many species have narrow habitat requirements, chironomids are valuable environmental indicators, suitable for biomonitoring of freshwater ecosystems. Past studies have shown that DNA barcoding is very useful for species identification and delimitation in the taxonomically difficult Chironomidae and an excellent tool for the association of unknown and cryptic life stages. However, our analysis of more than 2300 specimens belonging to 90 genera has also revealed a number of unidentifiable barcode clusters within this family. The result from Neighbour-Joining analysis of these sequences distinguishes 489 clusters that can be considered species, but we have only been able to identify 258 of these species based on morphology. As the project proceeds and additional reference collections are examined, the number of identified species will undoubtedly increase. Nevertheless, our DNA barcode data currently indicate at least seven cryptic species complexes and numerous new species. Our results also point to a few new cases of synonymy and rare instances of horizontal gene transfer. In conclusion, DNA barcoding can be used to pin-point groups that require detailed taxonomic assessment or revision, aiding our understanding of species diversity in the family Chironomidae.

DNA BARCODING OF BUTTERFLIES AND SKIPPERS FROM WESTERN GHATS, INDIA

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In this study, utility of mitochondrial gene cytochrome oxidase I (COI) as a DNA Barcode was tested. We have generated COI sequences for about 100 morphologically identified species of butterflies and skippers from Western Ghats of India. Of these sequences, about 70 % correctly matched with sequences of the species submitted under same name in the BOLD database from different parts of the World. Other sequences are additions to the DNA barcode library. Our study reveals that, there is distinct barcoding gap between intraspecies and interspecies divergence. Sequences from 2 or more specimens belonging to the same species always formed monophyletic clade. Mean intraspecies divergence (K2P model) observed was 0.28% and threshold of 2.8% (the 10× rule) was indeed very successful in discriminating the species. About 50 specimens of a pierid *Eurema hecabe*, a species known to be infected with *Wolbachia*, sampled from distant populations, revealed many haplotypes. Thus unique DNA Barcode is unlikely in a widely distributed species. Even then all group together as a single clade and matched well with sequence of *E. hecabe* in the BOLD database.

However, in a lycaenid, *Talicada nyseus*, with systemic infection of *Wolbachia*, a universal primer (LepF1-LepR1) led to the amplification bacterial COI instead of butterfly gene. As many insects harbor *Wolbachia*, such problems may challenge the universality of DNA barcoding for species identification. On comparison of our sequences and database sequences, in some cases, we found that genetic divergence within species to be in the range of 3.6 to 6 %.

Overall, this study funded by Department of Biotechnology, Government of India, indicates that such additional tool of DNA Barcoding and sequence database will be useful in identification of butterflies and skippers (even when only a small piece of tissue is available).

PERSPECTIVES OF ITS2 AS DNA BARCODING IN BLOWFLIES

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The internal transcribed spacer 2 (ITS2) is a small non-coding region located inside the ribosomal DNA cluster between 5.8S and 28S genes. ITS2 is claimed to be a reliable molecular marker for phylogenetic reconstruction and species identification, especially because of the compensatory base changes (CBCs) that occur between species. Recently, ITS2 has been suggested as an alternative barcode molecule, either to provide species identification in cases where COI is inconclusive or to provide additional information from a nuclear molecular marker. In this context, we cloned and sequenced the ITS2 region of 33 Calliphoridae species (Diptera: Brachycera) and, for one species, Cochliomyia hominivorax, we have sequenced the ITS2 region from 32 individuals from ten different populations, comprising the current geographical distribution of the species. The p genetic distance between different species varied from 0,007 for species of the same genera (including C. macellaria and C. hominivorax) to 0,175 for species of different subfamilies. Until now, nucleotide variation was not detected between different populations of C. hominivorax, with sequences varying only in length (from 351 to 359 bp). The indel events underlying the length variation are located mainly in the fourth domain, which is the most variable both in sequence and secondary structure. Regarding the CBCs, they always appear between species, but none was found among C. hominivorax populations. These results suggest that ITS2 could be a reliable molecular marker for species identification, corroborating to the recent suggestion of its use as an alternative barcode molecule.

Poster Abstracts

Insects & Terrestrial Arthropods

A tRNA BASED PRIEMR COCKTAIL FOR MITOCHONDRIAL COI BARCODING OF HEXAPODA

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DNA barcoding uses a 650 bp segment of the mitochondrial cytochrome c oxidase I (COI) gene as the basis for an identification system for members of the animal kingdom and some other groups of eukaryotes. PCR amplification of the barcode region is a key step in the analytical chain, but it sometimes fails because of a lack of homology between standard primer sets and target DNA. In this study we describe a primer design strategy which enabled resolution of this problem for the most diverse group of animals - the Hexapoda.

We began by analyzing the gene arrangements for all arthropod mitochondrial genomes in the Mitom database. This analysis revealed that the tRNA-W gene was invariably located within 200 bp upstream of CO1 gene, while sequence alignment showed two distinct groups of tRNA-W with high internal homogeneity. Two forward PCR primers were developed to best represent these two groups and were combined with a standard reverse primer (LepR1) to produce a cocktail which was tested against 141 species representing 126 different families of Hexapoda, including groups such as scale insects that invariably fail to amplify with standard primers. This cocktail generated 111 amplicons, allowing characterization of the usual primer binding region in COI 5' as well as the barcode segment. These cases of success included groups such as the scale insects whose unusual nucleotide sequence at the regular forward primer binding site provide an explanation for past failures in amplification. Later performance tests on more taxa revealed that the new cocktail amplifies CO1 for nearly all hexapods which fail to amplify with regular barcode primers. We suggest that the design of primers that bind in highly conserved gene regions upstream of COI will be a useful strategy in resolving problems in amplification.

DNA BARCODING THE HEMIPTERA

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Although DNA barcode coverage has grown rapidly for some insect orders, prior work on the Hemiptera has focused on just two families (Aphididae, Adelgidae) of the approximately 160 families belonging to this order. This study sought to broaden coverage, largely through the analysis of material in museum collections. DNA was extracted from 1700 specimens with an average age of 12.2 years. Barcode records over 500 bp were recovered from 1173 of these specimens providing coverage for 374 species representing 33 families and 190 genera. Sequences divergences (K2P distance) between congeneric species averaged 9.99%, a value which was 11-

fold higher than the mean intra-specific variation. Moreover, the intra-specific divergence value was likely inflated by the presence of species overlooked by current taxonomic treatments because several 'species' showed divergences greater than 2.0%. Other clusters included individuals assigned to several species, suggesting the need for more detailed morphological and molecular studies. Taken collectively, the present results suggest both the feasibility of creating a comprehensive barcode library for the Hemiptera and its value in both revealing taxonomic situations worthy of deeper analysis and in creating an effective system for identifying species in this group.

MORPHOLOGICAL ANALYSIS OF *ANTAEOTRICHA* SPECIES UNITS THAT ARE DIFFERENTIATED BY DNA BARCODING. (LEPIDOPTERA: GELECHIOIDEA: STENOMATINAE)

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Gelechioidea is the least known of the three extremely specious Lepidoptera superfamilies that largely constitute ¨Microlepidopteran¨ Ditrysia. Although delimited as a monophyletic group, the family and subfamily delineations are still being debated. The generic boundaries are not well defined and in some cases genera have become just depositories for somewhat similar species. One of the genera that, despite doubts about nomenclatural formalities and lacking a phylogenetic analysis, has been treated as a solid unit through the years is the genus *Antaeotricha* (erected by Zeller in 1854). This is a genus with close to 500 species in the neotropics and with little natural history information, with the exception of a few agriculturally important species. The number of described or underscribed *Antaeotricha* in Costa Rica is unknown. The plasticity of patterns, morphological variation, and sexual dimorphism make it very difficult to both delineate and identify species with certainty.

Using the results of the DNA barcoding of 800+ reared *Antaeotricha* from Area de Conservación Guanacaste (ACG), northwestern Costa Rica, we were able to differentiate more than 60 species units. Yet more ACG species were added when DNA barcodes from wild-caught *Antaeotricha* (the BioLep project to inventory and barcode the entire ACG) were included in the NJ tree.

A phylogenetic analysis using morphological characters was performed on the species units (males and females) revealed by DNA barcoding. Information on host plants, parasitoids and microgeographic distribution was then mapped onto the morphology-defined and DNA-defined units, to confirm or question the validity of these units.

While this is not a revision of the entire genus *Antaeotricha*, we feel that the characters discovered and species analyses constitute a platform for eventual clarification of the genus and the species groups within it.

Poster Abstracts

Insects & Terrestrial Arthropods

USING BARCODES TO IDENTIFY CATERPILLARS AND MOTHS OF THE YUCATAN PENINSULA: THE FIRST APPROACH FOR LEPIDOPTERA IN MEXICO

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Since 1990 the zoology museum of El Colegio de la Frontera Sur (ECOSUR), started to form the reference collection about the fauna of the Yucatan Peninsula. The biggest collection is the butterflies' one, with nearly 70,000 specimens but little is known about the moths, the major group of Lepidoptera in the world.

The knowledge of this group in Mexico is limited and the situation is worse about the larval stage of whole Order, mainly because the lack of specialists in the country working on this group. As alternative, using morphology and the barcoding to compare with different projects available on BOLD, we got the identification of caterpillars and moths' species.

At this moment, there are 3,194 specimens barcoded of which 66% are caterpillars. Until now we have 73 species among 10 families (Arctiidae, Crambidae, Geometridae, Hesperiidae, Lasiocampidae, Limacodidae, Mimallonidae, Noctuidae, Saturniidae and Sphingidae) of larval stages identified trough barcodes and 137 species of adults identified, including four endemic species to Mexico (*Spaeromachia gaumeri*, *Eacles imperialis quintanensis*, *Ptiloscola wellingi* and *Rothschildia lebeau yucatana*). It is probably that the subspecies *E. imperialis quintanensis* and *R. lebeau yucatana* would be raised to species level as the barcoding shows evident separation with the species E. imperialis and *R. lebeau*. However, further work is necessary to confirm this.

Also like in previous works, barcoding made possibly the identification of criptic species even in larval stage. There are three groups of the *Astraptes fulgerator* complex identified by barcoding and compared with Janzen's work. Two of these groups are new for the complex and it is necessary to find the adult stage of these caterpillars through barcoding.

In Mexico, this is the first work barcoding Lepidoptera and it is important to continue working with, to keep growing the barcode reference database in order to improve the potencial of identification by BOLD.

Poster Abstracts

Insects & Terrestrial Arthropods

DNA BARCODING TO DISTINGUISH SPECIES OF INDIAN ORTHOPTERA

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The identification of Orthpotera on the basis of morphology alone can be problematic due to seasonal colour patterns; mimicry as well as the species are far more numerous. The Orthopteran fauna is poorly represented in Fauna of Indian region especially from Western Ghats. The use of short DNA sequences from standardized region of the genome also referred to as 'DNA barcodes' has been proposed as a tool to facilitate species identification and discovery. This has been advocated and successfully used in groups of animals like amphibia, fish, beetles etc. Studies on identification of Orthoptera of Western Ghats (India), one of the biodiversity hotspots from Indian subcontinent using molecular tools, especially by DNA barcoding are lacking. Here we have used cytochrome oxidase subunit 1 DNA barcodes to effectively discriminate amongst species in four Orthoptera families Acrididae (25), Phasgonuridae (20), Gryllidae (5) and Gryllotalpidae (1) from Western Ghats. We found that all the morphologically distinct species that were sampled so far have distinctive cytochrome oxidase barcode. Our results suggest that the Orthoptera from the Western Ghats are poorly represented in molecular databases such as Genebank and BOLD. Thus our study adds new sequences to the existing vast data available for Orthoptera. This will help defining phylogeny among oriental as well as world Orthoptera. Our results suggest that the Orthoptera from the Western Ghat have distinct genotype than that of the already reported in the database till date. These results also show that DNA barcoding can significantly aid species identification of Orthpotera from India. as Dociostaurus decisus Walker, Aswatthamus cylindricus, Gryllus thalassinus, Edaleus nigrofasciatus, etc.

DNA BARCODING AND MINI-BARCODING FOR MOLECULAR IDENTIFICATION OF DIPTERA

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We tested the performance of DNA barcoding in Diptera by considering an alignment of 4,272 DNA barcodes involving 345 species from 75 genera. The objectives of this study were to 1) compare the performances of different identification criteria, 2) evaluate if different fragments of the COI barcode region provide comparable information, and 3) investigate relationships between barcode length and identification success of Diptera. In contrast to previous studies, we performed simulations under a "best case scenario" *viz.* by providing for each query one or more potential

conspecific matches in the reference database. Intra- and interspecific genetic distances were largely overlapping with 26.43% of pairwise comparisons shared between the 95% percentiles of distributions. Despite there was no clear "barcoding gap", the Best Match (BM) and Best Close Match (BCM) criteria yielded relatively high proportions of correct identification (BM=0.945, BCM=0.944) and performed better than Neighbor-Joining Tree (NJT=0.490). Different regions of the barcode fragment provided comparable information and mini-barcodes of 220bp still yielded substantial correct ID proportions (BM = 0.906 ± 0.016; BCM = 0.905 ± 0.016). These results support the BM and BCM methods *per se*. Nevertheless, the application of DNA barcoding in Diptera is currently limited by the low number of barcoded taxa. The lack of DNA barcodes for the overwhelming majority of the described Diptera species implies a high probability of making Type II errors (i.e. incorrect identification for queries without conspecifics in the reference database). Conversely, the probability of Type I error (misidentification of queries with conspecifics in the database) is relatively low (approximately 5% in our simulations). These considerations suggest that DNA barcoding may not be a foolproof method for the molecular ID of Diptera while it could be effective under well-defined conditions, where only a limited number of well characterised taxa are to be distinguished.

BIODIVERSITY OF TURKEY, DNA BARCODING OF THE FLORA AND BIRD FAUNA IN ANATOLIA: PROJECT OVERVIEW.

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Anatolia has a very high biodiversity. It is especially rich in endemic plant and animal species due to its geographic localization as a bridge between Africa, Europe and the Asian plate, its topographical features include low land and high land plateaus, volcanic and Line Mountains, wetlands, marshlands, deciduous, coniferous forest, semi desert area etc. and differing micro and macro climatic regions.

The assessment of biodiversity is one of the major aims in many countries of the world. In the last years, DNA barcoding is becoming a very powerful tool in systematic biology and biodiversity research, because it allows the identification of the species using genetic material. DNA barcoding analyses short genetic sequences from a standard part of the genome in the same way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code. DNA barcoding is a standardized approach for identifying animals and plants relying on minimal sequence information of DNA. Barcoding will help many biologists to quickly and cheaply recognize known species and retrieve information about them, and will speed up the discovery of the millions of species yet to be named. These are the reasons why we prefer the DNA barcoding for the assessment of biodiversity in Turkey. This project is a pilot project in which the details will have to be worked out with a focus on Anatolia.

MITOCHONDRIAL GENOME: THE BIOMARKER FOR INDIAN BIODIVERSITY

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India predominantly depends on agriculture allied with animal husbandry and fisheries which date backs to 5000 years of incessant civilization and endowed with a wide range of large varieties of flora and fauna suitably adapted to agro-climatic conditions. The eastern region (including Sunderbans) and the north-eastern region (including Eastern Himalayas) of India having a wide variation in climates ranging from extreme hot to cold conditions grew as biodiversity hotspots of the breeds/strains of flora and fauna. The biodiversity and ecosystem stability are threatened by

invasive alien species. In this scenario, investigation of the biodiversity and the genetic uniqueness based on mitochondrial DNA variation along with its identification, characterization and patenting of the unique genetic traits of animals and plants in this biodiversity hotspot is highly demanding. Sequence diversity in a 650 bp region near the 5' region of mitochondrial Cytochrome oxidase subunit1 (COI) gene provides a strong species level resolution for varied animal groups. We have already deposited DNA barcode sequences of several animals including Royal Bengal Tiger (National animal of India), Indian domestic Cat, Oysters, Shrimps, Cattle and Buffalo, etc. DNA barcodes of different groups of Fishes (including Ornamental fish), turtle and tortoise, mammals, birds, medicinal plants and other species found in North East Himalayan biosphere are underway. We have also performed extensive analysis of mitochondrial d-loop of different breeds of Sheep and Black Bengal Goat found in the eastern and north eastern part of India. Our research revealed that mitochondrial d-loop sequence can be an invaluable tool for breed specific barcoding with wide phylogenetic implications.

Poster Abstracts

Marine Barcoding

GENETIC HOMOGENEITY AND CONNECTIVITY AMONG POPULATIONS OF SPECIES *MUNIDOPSIS GEYERI* AND *ALVINOCARIS MURICOLA* OF THE ATLANTIC EQUATORIAL BELT

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Munidopsis geyeri (Decapoda: Galatheidae) and *Alvinocaris muricola* species (Decapoda: Alvinocarididae) are benthic crustacea that coexist it sites with chemoautotrophic activity. The two species display an amphiatlantic distribution suggesting a connectivity of the metapopulations within the Atlantic Equatorial Belt region. Understanding the distribution strategies of the galatheid-caridean species pair is relevant to the study of morphological differentiation and genetic isolation of species adapted to chemosynthesis with different types of reproductive strategies, larval development, and sizes. The results of our ongoing study will try to answer the question of how similar these species are along a longitudinal gradient at a regional scale (within the Gulf of Mexico) and a larger amphiatlantic scale and describe the molecular variability of these species based on partial sequences of the mitochondrial genes 12S, 16S and COI extracted from individuals of the species *M. geyeri* and A. *muricola* obtained from populations of different locations within the Equatorial Atlantic Belt region. Tissue samples were obtained from specimens deposited in collections (CNCR-Mexico, MNHN and IFREMER-France, NMNH and the Pennsylvania State University -USA). These specimens were collected within a depth range of 1300- 4000m under both abyssal and chemosynthetic conditions in the Gulf of Mexico (GoM) (asphalt volcano site in the Southern GoM and Alaminos canyon, Atwater valley and Florida Escarpment in the Northern GoM) and different locations in the Atlantic: Caribbean (Haiti) and Gulf of Guinea (REGAB site). The analysis has been done by comparing first within the Gulf of Mexico basin among continental slope and abyssal plain sites without chemosynthetic activity (seeps). Our preliminary results allow us to recognize the existence of connectivity between populations in the region based on no genetic differentiation. A potential connectivity based on the existence of populations that have not been sampled yet but lie in intermediate sites and are prone to settlement of larvae between existing, sampled populations is herein suggested and supported with other analysis.

SPECIES IDENTIFICATION IN THE THALIACEA

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The Thaliacea are a group of approximately 70 species of holoplanktonic, gelatinous invertebrates, consisting of 3 major lineages: the Salpida (salps), Pyrosomatida (pyrosomes), and Doliolida (doliolids). Many nominal species appear to be broadly distributed throughout the world's oceans,

although for some, variation in morphological features has been documented. Despite their prominence in many ocean ecosystems and ecological role in transferring organic material from the surface to the deep sea, thaliaceans are relatively little studied, in part due to difficulty in handling and identification of samples. Obtaining intact specimens can be difficult as the organisms are readily damaged in sampling gear, many thaliaceans have morphologically distinct life history stages that make species discrimination challenging, and there are few experts capable of accurate species identification. The goal of our work is to find and describe a species-specific genetic marker that can act as a barcode to facilitate research in thaliacean biodiversity, evolution, and ecology. Because mitochondrial genes typically used in barcoding do not readily amplify in thaliaceans, we are pursuing amplification of the internal transcribed spacer regions of the ribosomal DNA (ITS). Here we examine the inter- and intra-specific variation in thaliacean ITS sequences and discuss its utility as a thaliacean species barcode.

DNA BARCODING OF FREE-LIVING MARINE NEMATODES USING 28S rDNA GENE

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In this study, we evaluated the diversity of free-living marine nematodes using complementary morphological and DNA Barcoding data. The samples were collected in two localities in the Gulf of California Mexico (Gulf of Santa Clara and La Paz Baja California Sur). Using standard techniques of molecular biology the nuclear 28S rDNA gene was amplified and sequenced. Morphological identification was done microscopically using morphological identification keys. We identified 83 individuals in 55 morphospecies, from these, only 47 could be taxonomically determined in 37 genera and 15 families. DNA barcoding data was obtained from 27 individuals in 21 morphospecies. Their morphological identification was congruent with the DNA sequences. Identical or very similar sequences corroborated the morphological identification of organisms from the same morphospecies (eg. *Oncholaimus* sp n = 4, *Viscosia* sp n =2 and *Chromadora* sp1 n=2). Maximum Parsimony and Neighbor Joining produced different but concordant phylogenetic reconstructions, in which several lineages were strongly supported (non-parametric bootstrap > 90%). In conclusion, our results suggest that the identification of free-living marine nematodes will benefit from a combination of molecular and morphological data. DNA barcoding of these organisms will allow a better understanding of their diversity, including the assessment of cryptic diversity.

Poster Abstracts

Marine Barcoding

DNA BARCODING MARINE FISHES FROM KAKINADA COAST, (A.P.) INDIA

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Mitochondrial DNA, Cytochrome oxidase-1 gene sequences were analyzed for species identification and phylogenetic relationship among the very high food value and commercially important Indian Marine fish species. Sequence analysis of COI gene very clearly indicated that all the one hundred and fidty eight fish species collected from Kakinada coast, Andhra Pradesh, India fell into fifty five distinct groups, which are genetically distant from each other and exhibited identical phylogenetic reservation. All the species selected under study exibits high food value and export potential as a processed food. All the COI gene sequences from twenty eight fishes provide sufficient phylogenetic information and evolutionary relationship to distinguish these species unambiguously. This study proves the utility of mtDNA COI gene sequence based approach in identifying fish species at a faster pace.

DNA BARCODING OF MARINE PLANKTONIC OSTRACODS: GOLD STANDARD DATABASE FOR AN 'INVISIBLE' GROUP

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Marine planktonic ostracods or "seed shrimp" (Phlyum Arthropoda, Subphylum Crustacea, Class Ostracoda) are good exemplars of the problem of accurate species identification of the marine holozooplankton assemblage. The group is diverse, with over 200 described species in two subclasses and four orders. Most species are relatively small (adult sizes 0.5 – 5.0 mm). A representative oceanic zooplankton sample from mid-depth (1000 m) and mid-latitudes (within 40° N and S) will contain 30-40 species. Ostracods are numerically abundant and ecologically important in most oceanic regions and throughout the water column, often ranking second only to another crustacean group, the Copepoda. Yet ostracods are a nearly invisible component of pelagic assemblage: they are routinely overlooked by plankton ecologists; only 4 or 5 researchers routinely identify species.

The Census of Marine Zooplankton (CMarZ) has brought together taxonomists and DNA barcoders to work toward a global gold-standard barcode database for ostracods. CMarZ cruises to the Sargasso Sea (Northwest Atlantic) and eastern Atlantic Ocean were carried out to sample from the

surface to 5000 m depth. Living specimens were examined immediately after capture, with 104 species identified. DNA barcoding was performed at sea in a ship-board sequencing laboratory. DNA barcodes (a ~600 base-pair region of mitochondrial cytochrome oxidase I) were determined for 222 specimens of 84 species. For most taxa, intraspecific variation of mtCOI was low (0% to 4%), while interspecific was large and diagnostic of species (15% to 58%). Comparison between the western and eastern Atlantic collections revealed evidence of cosmopolitan species, as well as evidence for a number of cryptic species. Taxonomic questions remain for several systematic complex and morphologically ambiguous groups. Further taxonomic and genetic investigation will be necessary to determine DNA barcodes for these groups.

MORPHOLOGICAL AND MOLECULAR APPROACHES TO ASSESS MARINE NEMATODE DIVERSITY: IMPLICATIONS FOR DNA BARCODING

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We used morphological and molecular approaches to evaluate marine nematode diversity (order Enoplida) in sand samples collected in the Gulf of California (4 sites) and the Pacific coast of Baja California (3 sites), in Mexico. Two nuclear ribosomal genes, 18S and 28S (D2D3 domain) were amplified using standard molecular techniques. We identified 22 possible morphological species from six families, some of which may be new to science. Thoracostomopsidae and Oncholaimidae were the most diverse families with seven and three genera, respectively. *Mesacanthion* (Thoracostomopsidae) was the most common genus, being found in five localities: two in the Gulf of California (*M*. sp1 and *M*. sp2) and three in the Pacific coast (*M*. sp3, *M*. sp4 and *M*. sp5). Morphological and molecular data supported the differentiation between these five morphotypes. We produced 19 and 20 unique sequences (MOTU) for the 18S and 28S genes, respectively. Molecular data were congruent with morphological identification since each species was represented by a single MOTU for both genes, and showed no intraspecific genetic polymorphisms. In addition, morphological-genetic contrasts revealed patterns of ontogenetic variation (between juveniles and adults) and phenotypic plasticity (between adults), stressing the importance of an integrative approach for biodiversity assessment. Although, 18S and 28S tree topologies were congruent (ILD test, p>0.05), MOTU divergences were much higher in the 28S gene. Moreover, 28S provided a better phylogenetic signal, grouping the genus *Rhabdodemania* with *Bathylaimus* and not with the Oncholaimids. The D2D3 domain may have better features for DNA barcoding in marine nematodes than 18S, mainly for differentiating closely related and cryptic species. Finally, we suggest that an integrative approach will improve the assessment of marine nematode diversity making for a stronger nematode taxonomy.

Poster Abstracts

Meso-American Symposium

DNA BARCODES OF TOPOTYPES MAMMALS OF MEXICO I: NORTHWEST MEXICO

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A topotype is a specimen collected in the same locality that the type specimen and that is presumed belongs to the same species, thus the topotype has important implications in the recognition of the species. It is considered that the topotype belongs to the same population of the type; therefore, they have the same taxonomic attributes like coloration, morphology, size and forms, and it is also probable that they have the same haplotype. The topotype has the advantage of being a fresh tissue, recently collected, thus the sequences are better in quality than the type specimen which is old and is subject to the limitations of the preservative. For this reason, the topotype specimen is the best alternative, mainly when the type specimens are not available. In this project we will obtain the barcodes of mitochondrial DNA (COI) for at least 150 topotype taxa of terrestrial mammals from the northwest of Mexico. These specimens will be integrated to the database of BOLD system. The project will contribute with information applicable to the identification of species of Mexican mammals, mainly cryptic species and those considered under synonyms. The specimens to study are housed in the Collection of Mammals of the Biological Research Center of the Northwest, Mexico (CIBNOR).

ANALYSIS OF THE ENDEMIC AND ENDANGERED SPECIES OF HETEROMYS IN MEXICO

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Heteromyidae rodents are very important species in the tropical areas of the American continent. The genus Heteromys is restricted to the tropical forest from Mexico to the north of Central America. The genus has had many taxonomical changes in the last years, including the description of new species. In the case of Mexico four species have been recorded. One of them (Heteromys goldmani) with a very restricted distribution and which have been considered as subpopulation of the species with wide range. The restricted species Heteromys nelsoni is only found in high elevations of the mountain range of the southern part of the state of Chiapas, Mexico. Both species have a strong human pressure in the modification of its habitat and H. nelsoni was only known from two mountains, Cerro Mozotal, in Chiapas and Volcán Tajumulco, in Guatemala. I used the COI to help in the identification of other third population of H. nelsoni in Chiapas and made an analysis of the relation of the two endemic species H. nelsoni and H. goldmani in relation to the two wide distributed species H. desmarestianus and H. gaumeri. The molecular analysis shows the validation of the four species and a very low variation in the number of haplotypes in the restricted distributed species.

Poster Abstracts

Meso-American Symposium

SPECIES DIVERSITY IN A GROUP OF PARASITOID WASPS FROM THE CHAMELA-CUIXMALA BIOSPHERE RESERVE

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Hymenopteran parasitoids are among the most diverse groups of insects, of which the Braconidae represents the second largest family. Braconid wasps mainly parasitize larvae of different insects orders, playing an essential role in almost all terrestrial ecosystems. Unfortunately, only a small proportion of all braconid species has been described, this due to their enormous diversity, the lack of specialist taxonomists and the presence of a high number of cryptic and pseudocryptic species. Here we show an ongoing investigation that makes use of DNA barcodes to discover the species diversity of the braconid subfamily Doryctinae in the Chamela-Cuixmala Biosphere Reserve in Mexico, a region mostly composed of dry forest. This subfamily predominantly comprises idiobiont ectoparasitoids of xylophagous and bark boring Coleoptera larvae, and contains various speciose, morphologically conserved taxa. Our study will generate barcode sequences of about 1,000 doryctine specimens from over 30-50 morphospecies, and we expect that it helps to uncover a number of cryptic/pseudocryptic species that would not be distinguished using traditional taxonomy alone. The material collected will also help to create a hymenopteran tissue/DNA collection at the Instituto de Biología, UNAM.

DNA BARCODING, ESSENTIALISM AND INSTRUMENTALISM: A REEXAMINATION

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Recently, two philosophically-oriented viewpoints against DNA barcoding have been presented, namely, that (a) DNA barcoding entails an essentialist view of species in biology (Rubinoff, Cameron and Will 2006) and that (b) DNA barcoding implies an instrumentalist perspective in systematics (Rieppel 2007) and therefore in taxonomy. Noting the obvious, common conclusion of these two stances –i. e. that DNA barcoding should not be supported because Essentialism and Instrumentalism are opposed to the modern, Scientific Realist view of evolving biological entities– I will argue that while claim (a) loses validity when confronted with recent historiographic work (Amundson, Müller-Wille, Winsor) which has debunked the 'Essentialism Story', claim (b) insufficiently acknowledges the distinction between DNA barcoding and 'DNA taxonomy'. I think that this reconsideration is useful to improve the conceptual grounding of 'integrative taxonomy' (IT) *sensu* DeSalle, Egan and Siddall (2005), where molecular data are legitimately used to produce and/or justify 'species hypotheses', together with other sources of systematic evidence in a multiple-step inferential process. In this regard, I will further argue that the epistemology of IT, in which DNA barcoding plays a role, suggests the abandonment of naïve, outdated Realist stances

on the ontology/metaphysics of biological species, historically associated to the anti-taxonomic strand of the 'Modern Evolutionary Synthesis'.

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AN ALL TAXA BARCODE INITIATIVE FOR THE BIOTA OF A BIODIVERSITY HOTSPOT IN VERACRUZ, MEXICO

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The Los Tuxtlas region, in the tropical humid southeastern portion of the state of Veracruz, Mexico, is noted for its high biological diversity. The Los Tuxtlas Biological field station (EBTLT), owned and operated by the Institute of Biology of the Universidad National Autónoma de Mexico (IBUNAM), has been pivotal for research on various aspects of taxonomy and systematics, functional, organismic and population biology, environmental ecology, and ecological restoration for over 40 years. EBTLT is located within one of the core zones of Los Tuxtlas Biosphere Reserve, which includes at least eight different vegetation types, and many endemic taxa. The biota in the station and its surroundings is relatively well known. The dominant vegetation there is tall evergreen rainforest, and within the 640 ha UNAM reserve over 900 species of vascular plants have been documented, as well as 400 species of birds, 126 of mammals, 118 of reptiles, and 46 of amphibians. Some groups of invertebrates have also been studied. Voucher specimens for the majority of the species documented are deposited in the reference collections on site or in the national collections of IBUNAM. Los Tuxtlas is particularly attractive for tropical biologists since it is one of the few remaining lowland rainforest areas at this northern latitude. The field station and its facilities are ideal for conducting DNA barcoding projects on many taxa. Recently, a pioneer research project focused at barcoding the tree flora of EBTLT has been initiated, and work on the first 200 rainforest tree species is already in course. With this iniciative we hope to get participants that have collected specimens in the area to barcode their species, and new participants to become involed. A special web page will be developed for the initiative.

Poster Abstracts

Pathogens, Disease Vectors & Parasites

CONVERGENCE TO MEATZOAN MITOCHONDRIAL PRIMER TARGETS BY MARINE MICROBES

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The goal of DNA barcoding initiatives is to develop a global standard for the identification of life. Two factors critical for their success are the accurate identification of reference specimens and the validation of DNA sequences associated with those specimens. Employing commonly used DNA barcoding primers, we generated sequences for several marine invertebrates and obtained matches from standard databases. In addition to closely matching a variety of putatively metazoan barcode sequences as expected, a variety of bacterial sequences also yielded highly significant e-values. More detailed phylogenetic comparison to genomic cytochrome c oxidase subunit I sequences demonstrates that many barcode sequences, heretofore presumed to be of animal origin, are actually bacterial contaminants. Accidental amplification of cytochrome c oxidase subunit I sequences from marine Alteromonadales and Vibrionales has been facilitated by the bias in nucleotide composition predicted for psychrophilic microbes. The priming target sequences in several cold-adapted *Gammaproteobacteria* provide a better match to standard barcoding primers than do many animal mitochondrial genomes to the extent that these microbes may be preferentially amplified at the liberal annealing temperatures used when barcoding animals. We recommend that marine animal DNA barcodes first be validated against the growing availability of microbial genomic sequences, and that stand-alone barcoding databases take aggressive measures to denote probable contaminants in a manner not easily accomplished in public databases.

DNA BARCODING OF INSECT VECTOR-PATHOGEN RELATIONSHIPS: A CASE STUDY OF *OROSIUS* (HEMIPTERA: CICADELLIDAE)

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Orosius Distant (Hemiptera: Cicadellidae) is a small genus of leafhoppers of economic importance in Africa, Asia, the Pacific and Australia. At least half of the approximately 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 of 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 were used to group specimens into distinct clusters using a mixed Yule-coalescent modelling procedure. These clusters were compared with morphospecies clusters identified through male genitalic characters. Our study doubles the known species diversity of the genus and clarifies the identity and distributions of the species.

We have developed diagnostic tools to be used as a standard for future investigations of leafhopper-phytoplasma species associations. The implications of newly discovered cryptic species for studies of plant disease transmission are significant.

SPECIES OF THE *BULINUS TRUNCATUS/TROPICUS* (GASTROPODA: PLANORBIDAE) COMPLEX AND THE DNA BARCODING METHOD

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Bulinus sp. (2n = 36) is a diploid freshwater snail found in Cameroon crater lakes; it belongs to a group of medically important freshwater snails. Some members (*Bulinus truncatus*, *Bulinus tropicus*) of this group had been reported to be involved in the transmission of parasites (*Schistosoma* sp. and *Calicophoron microbothrium*) to human and livestock in tropical Africa. Yet, understanding of the evolutionary identity of the diploid snail such as its phylogenetic position and the genetic divergence among populations, remains limited. In this study, we constructed the molecular phylogeny of *Bulinus* sp. using sequences of mitochondrial cytochrome oxydase subunit 1 (CO-1, 365 nucleotides). Partial sequences of CO-1 were obtained and genetic divergences between populations estimated after the alignment of 365 nucleotides from each studied population. The lack of deep molecular divergences between populations of *Bulinus* sp. from western Cameroon crater lakes may indicate that they belong to the same lineage; therefore, it implies that diploid *B. truncatus/tropicus* complex snail-like in Cameroon share a common ancestor. The CO-1 of the three studied populations of *Bulinus* sp., clustered together with other diploid pan-African representatives of the *B. truncatus/tropicus* complex, showed little evidence of genetic similarities. A barcoding approach based on sequence of the cytochrome oxydase subunit 1 as defined by the Asmit primers is proposed. In conclusion, COI gene is a good marker to discriminate between species of the *Bulinus truncatus/tropicus* complex, compared to the morphological marker.

Poster Abstracts

Pathogens, Disease Vectors & Parasites

BIODIVERSITY, INDEGENOUS KNOWLEDGE AND THE ISSUE OF BIOPIRACY: THE RELEVANCE OF DNA BARCODING AND DA

ONYIA, C.O.(1), Solomon, B.O.(1), Ezebuiro, C.N.(1), Okujagu, T.F.(2), Otigbu, A.C.(1), Sadiq, H.Y.(1) & Madu, J.(1)

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Many of the developing countries in the tropics are very rich in biodiversity; even though they are generally poor in technology, they apply their indigenous knowledge for sustainable utilization of these resources in the promotion of affordable healthcare delivery and food security. It becomes imperative that these resources be protected to develop the necessary confidence in trading of bioresources between locals and entrepreneurs. In addition, the issue of bio-piracy further corroborates the need for a global program on indigenous species identification and data security and management. Admittedly, this is a huge task, considering the expertise and financial resources involved. Fortunately, certain molecular techniques have elaborate advantages on such issues and have been demonstrated to have the ability to couple information technology in storing the enormous data from such a global survey. In recent times, DNA barcoding techniques have been used in many advanced countries to characterize and document indigenous plant and animal species, among other uses. The technique, which has been widely employed as a tool for basic biodiversity research, involves the use of a short, standardized gene sequence. DNA barcoding is advocated as a cost-effective tool for regulating trade and sale of endangered species, and many other species of concern to governments and communities. Because the technique uses DNA and not morphological features, it can identify various stages of the organism, including eggs, larvae, pupa, blood, tissue, processed or fragmented forms of an organism, such as slaughtered and butchered meat or processed powders and milled grains. The species-specific information is fed into a database for reference in the situations of doubt over taxonomic identity of plant and animal biodiversity in the covered regions. Knowledge of the original source of a biological property can strengthen or weaken claims over ownership of indigenous knowledge prior to further scientific research and development. Therefore, this paper examines the current arrangement of biodiversity conservation and benefit sharing, its problems and the role DNA barcoding technique can play in generating the requisite confidence and support from bioresources-rich but technology-poor developing regions of the world. In addition, it examines the place of indigenous knowledge in IPR issues and possibilities of using DNA barcoding to check biopiracy.

Malaria is one of the most devastating of human diseases, but there are actually almost five hundred described species of related parasites that use a wide variety of vertebrate animals throughout the world as their hosts and four or more families of dipteran insects as vectors. However, the identification and taxonomy of these parasites from samples taken from their vertebrate hosts can be challenging for several reasons. First, because of seasonal or immunological reasons, there can be a paucity of some of the life-history stages in blood samples. Second, variation in morphology of the parasites in different host species has been observed, but in many cases, new parasite species were described because of the use of a different species of

host. In addition, the identification of the insect vectors of these parasites has been a century-long problem as well, because of the need for complicated experiments to demonstrate vectoral competence. The use of DNA barcodes from the malaria parasites has great potential to alleviate all of these issues. Barcodes can facilitate the discovery and description of new parasite taxa, the linkage of parasite samples where only some life history stages are present or which are in different hosts and barcoding also shows promise for helping to identify the insects that may be transmitting the parasites. I have recently proposed a combined approach of both the morphological study of parasites and the deposition of thin-blood smear vouchers into collections along with mitochondrial sequence generation for all novel species of malaria parasites. Studies of other members of the phylum Apicomplexa, which also have complex life cycles, will also benefit from similar approaches.

Poster Abstracts

Plant Working Group

CONSERVED BLOCKS IN CATALYTIC DOMAIN OF CKX GENE: AS A HIGHER TAXA MARKER.

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In the numerous interactions between plants and soil, microorganisms the plant growth promoting substances (PGPS) are believed to be the reason for the evolution of land plants. Existing literature on rhizospheric interactions include considerations about the evolution of phytohormone (plant growth regulators - PGR) system. The patterns of phytohormones distribution, their native function and possible origin of hormonal regulation across the plant taxa are important in understanding the evolution of utilization patterns from PGPS into PGR. Out of the five classical phytohormones, cytokinins occur ubiquitously in green plants. Cytokinin oxidase/dehydrogenase (CKX) gene plays important role in controlling the level of cytokinins in plant tissues, and regulate the cytokinin dependent processes by irreversible degradation of the cytokinins, isopentenyladenine, zeatin, and their ribosides in a single enzymatic step by oxidative N6 side chain cleavage.

Availability of genomewide information on an increasing but still limited number of plants offers the possibility of identifying orthologues, or related genes, in diverse species and higher taxa with complex genomes. In an ongoing project we have used the recently described (COnsensus-DEgenerate Hybrid Oligonucleotide Primers (CODEHOP) PCR strategy for targeted and direct isolation of partial CKX gene from important Solanaceous members. Analysis of the four CKX sequences (Withania somnifera - FJ790124.1, Nicotiana tabacum cv. oosikappal - FJ872118.1 and FJ872120.1 and Solanum nigrum - FJ872119.1) in comparison with meta-data gives a focus on the taxonomical diversity of and its possible application as a marker of higher taxa above the level of genus in the envisioned 'tree of life'.

THE GENETIC DIVERSITY OF THE *GALANTHUS L.* SPECIES IN TURKEY

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Galanthus L., widely known as snowdrops, is a genus of bulbous monocotyledons, consisting of 19 distinct species. The genus is confined to Europe, Asia minor and the Near East. Morphological and molecular characteristics of *Galanthus* taxa, represented by 12 species (14 taxa) and one hybrid in Turkey, were investigated. Genetic diversity of the *Galanthus* species was studied using both nuclear and chloroplast markers. Nuclear ribozomal DNA intragenic spacer region ITS1, ITS2 and 5.8S rDNA and chloroplast *trn*L(UAA) intron sequences as well as the intergenic spacer between *trn*L(UAA)3' and *trn*F(GAA)5' genes were examined. Phylogenetic trees were constructed using the

sequencing data with various phylogenetic methods. This study clearly establishes the geographical distribution of *Galanthus* taxa populated within Turkey.

IDENTIFICATION OF PLANT SPECIES IN THE FAMILY RUTACEAE WITH THE USE OF DNA BARCODING

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To identify the species in the family Rutaceae, In this study, six potential regions used as DNA barcodes were tested (i.e. *trnH-psbA*, *matK*, *ycf5*, *rpoC1*, *rbcL*, and ITS2). Through the analysis of 301 samples in 194 species of 71 genus, the result shows that ITS2 region followed by *trnH-psbA* had the highest level of variation and the highest success rate of identifying species based on BLAST method. Therefore, ITS2 region was the most effective barcode for the identification of species in the family Rutaceae. However, *matK* region was tested not suitable for the species identification of this family due to its low PCR amplification efficiency. Moreover, most of the unidentified species belong to Citrus genus, and their subgenus divisions and current number of species were still unclear. In brief, ITS2 region was a suitable barcode for the identification of species in the family Rutaceae.

INTERNAL TRANSCRIBED SPACER 2 (ITS2) REGION IS A UNIVERSAL DNA BARCODE FOR GYMNOSPERMS

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DNA barcoding has been accepted as an efficient tool for the discrimination of animal species. However, consensus has not been reached as to which DNA region(s) should be the standard barcode for land plants. Here, we sought to determine a suitable barcode for gymnosperms by comparing seven potential DNA barcodes (*psbA-trnH, rbcL, matK, rpoB, rpoC1,* ITS and ITS2). Based on four criteria, including PCR amplification success, intra- and inter-specific genetic divergences, the "DNA barcoding gap" and identification ability, our tests suggest that ITS2 represents the most suitable region for DNA barcoding. To further evaluate the performance of ITS2 region, we also tested its ability in identifying a wide range of gymnosperm species using 888 plant samples. These 888 samples represent 500 species from 80 diverse genera of all families of gymnosperms. The

rate of successful identification with ITS2 was 73 % and 98% for the samples at the species and genus level, respectively. The results obtained from this study suggest that the ITS2 region be a universal barcode for gymnosperms.

SEARCHING FOR BARCODE REGIONS/SECTORS APPLYING FOR NEXT-GENERATION SQUENCING TECHNOLOGIES: A CASE FROM FERN GENUS DEPARIA

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The next-generation sequencing technology provides an efficient way for the establishment of metagenomic libraries, in which DNA characteristics could be applied to identify featureless or cryptic organisms. Although the idea of using metagenomic libraries to infer diversity of some taxa in ecosystems has been practiced, the criteria of DNA regions fitting this idea have been taken into less consideration of characteristics of future DNA barcodes. In addition to the limitation of sequencing read length, the next-generation sequencing technology has higher sequencing error comparing to the traditional sequencing technology; and the increase of sequencing errors from the next-generation sequencing technology consequently, results in misleading intra-species divergence and ambiguous boundary between intra- and inter-species divergences. Therefore, searching for region or sectors with the deepest "barcode gap" is needed.

Here, we focused on taxa belonging to a small fern genus, *Deparia* (Woodsiaceae). Within few proposed DNA barcode regions, we used the approach of sliding window to search for short sectors (200 bp), in which "barcode gaps" are shown with higher inter-species divergence (pi) than reevaluated intra-species divergence under the consideration of sequencing errors. We found that *matK* has highest proportion of regions/sectors with diagnostic "barcode gap" (more than 90 percent) while less than 10 percent of regions/sectors were in *rbcL*. Moreover, our analysis is the first report to identify "barcode gap" of *matK* in ferns providing the sequence characteristics of *matK* for future fern DNA barcoding.

USING TWO-LOCUS DNA BARCODES TO BARCODE A REGIONAL FLORA IN COSTA RICA

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The flora of the 110,000 hectare terrestrial portion of the Area de Conservacion Guanacaste, (ACG), northwestern Costa Rica, contains at least 6,000 species of angiosperms. Because of its high diversity, this flora provides a good model system for testing the effectiveness of the two plastid regions (rbcL, MatK) selected for plant barcoding for identification and discovery of cryptic species. Three specimens from each of 400 species (280 genera, 86 families), representing most of the angiosperm families in the ACG flora, have been barcoded. Standard protocols for plant barcoding generated rbcL sequences for 87% of the species, and MatK sequences for 69% of these

taxa, resulting in joint sequence information for just 65% of the indicated species but one or the other sequence for 98% of the species and 90% of the specimens. Because of its high PCR success and ease of alignment, rbcL contributes much taxonomic information, but its resolution was inadequate for species discrimination in 15% of cases of comparison of species. MatK offered higher resolution within many of these groups. Taken with one or the other sequence, only 2.5% of the species could not be identified with 100% certainty by their barcode. The mean genetic distance between congeners was similar for both markers (1.8%), but MatK showed greater variance (SE=0.10 vs. 0.05 in rbcL), aiding taxonomic resolution in those genera with deeper divergences between species. The barcode results revealed 10 cases of apparent splits within morphospecies reinforcing the possibility that plant barcodes can be used to disclose unnoticed taxa in regional species-rich floras.

DNA BARCODE FOR MEXICAN BAMBOOS (POACEAE: BAMBUSOIDEAE)

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In Mexico 36 native bamboo species have been reported and 15 are endemic, found in tropical vegetation and in montane forests. Bamboos are widely used, for example *Guadua aculeata* and *Otatea acuminata* are utilized in construction, *Otatea acuminata* is used in landscaping, *Rhipidocladum racemiflorum* for making arts and crafts. Among endemic taxa is *Olmeca*, a small genus with two species from southern tropical forests, which are listed in the Mexican red list of threatened species.

Plants of twenty five species are being cultivated as part of the national living bamboo collection at the Francisco Javier Clavijero Botanical Garden (Instituto de Ecología, A.C.).

The objective of this project is to barcode the Mexican bamboo species to allow easy identification of these taxa.

DNA sequences of the two genes selected as the plant barcode, the chloroplast loci *matK* and *rbcl*, will be obtained from individuals in the Clavijero collection as well as from plants newly collected. Several individuals will be sequenced to evaluate if these markers adequately discriminate at species level, particularly for congeneric species. In addition, collections of the project will increase the bamboo collection at the Clavijero Botanical Garden.

DNA sequences of the Mexican bamboos will contribute toward the discovery of overlooked species.

Poster Abstracts

Plant Working Group

A DNA BARCODE FLORA OF THE NORTHEASTERN UNITED STATES AND ADJACENT CANADA

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The flora of the northeastern United States and adjacent Canada is well studied. The most recent manual of the flora was published in 1991. The New York Botanical Garden has committed its staff to the production of a revised flora. In addition to documenting plant diversity and identifying important natural areas, this effort will build local capacity and interest in botanical science and conservation. Several genera of woody plants found in the region such as blackberries (Rubus), oaks (Quercus), and hawthornes (Crataegus) are notorious for defying classification using traditional morphological taxonomic methods. DNA barcoding may be able to provide additional characters for identification and insight into the genetic and dispersal history of these species. In this study, we have tested the effectiveness of two markers, matK and rbcL, in discriminating amongst the 223 species and 28 varieties of woody plants found in the region. The 251 taxa are arranged in 38 families and 78 genera. Specimens were collected from 20 localities dispersed throughout the northeast. We sampled an average of ten accessions per taxon. This sampling of closely related species coupled with high sampling density has allowed us to estimate intra- and interspecific variation at the barcode loci. We will focus our discussion on amplification success and discriminatory power of these two markers for the woody plants of the northeast.

DNA BARCODING OF THREE GENERA OF INDIAN ZINGIBERACEAE

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India is rich in family Zingiberaceae. Several species of this family are medicinal; their rhizome being traditionally used in indigenous health care systems such as Ayurveda, Sidha and Unani. The taxonomic identification system using morphological keys do not reliably identify plant parts like rhizome. However, accurate identification of genuine raw materials is vital in herbal sector in order to ensure batch-to-batch efficacy of herbal products. Our laboratory is engaged in developing a DNA barcode database of Zingiberaceae, aiming at providing a reliable biological identification tool to herbal industry. Here we present our results on barcoding 20 species of family Zingiberaceae: 10 species of genus Zingiber and 5 species each of Alpinia and Globba.

Twenty-eight primer combinations from published literature and 5 designed by the investigating group based on 9 chloroplast (trnL-F, rpl36-infA-rps8, rpoC, rpoB, accD, psb A-trn H, ndhJ matK and YCF) and one nuclear (ITS) targets were initially evaluated in 3 individuals each of Z. zerumbet, A. fax and G. schomburgkii. Nineteen combinations based on trnL-F, ITS, rpl36-infA-rps8, rpoC, rpoB, accD, psbA-trnH, ndhJ and matK targets repeatedly yielded single bands, and were gel-purified and sequenced. Analysis of sequence data using different bioinformatics tools revealed

less within-species and high between-species variation in rpl36-infA-rps8, rpoC, psbA-trnH, ndhJ matK sequences. Altogether, 77 accessions belonging to the 20 species were subsequently analyzed using these five selected targets. mat K sequences amplified by the primer combination designed by the investigators based on a consensus Zingiberaceae matK sequence resolved 16 out of the 20 spp. tested in a UPGMA dendrogram followed by rpl36-infA-rps8 (14/20), psbA-trnH (13/20), ndhJ (7/20) and rpoC (5/20). The study high lights the potential of matK in the molecular identification of Zingiberaceae.

TESTING THE FEASIBILITY OF DNA BARCODING IN A LARGE FAMILY, FABACEAE

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Fabaceae is the third largest family of flowering plants, with a large number of medicinal, economic and poisonous plants. However, traditional taxonomy can not meet the complicated demands of species recognition in Fabaceae. Thus a rapid and correct authentication is in great need to ensure safety and stable supplication of plants Family Fabaceae. Now we have tested six key claims of molecular taxonomy in Fabaceae. Three subfamilies of Fabaceae (Mimosoideae, Caesalpinioideae and Papilionideae) were all covered. Through six candidate promising markers, four coding chloroplast regions (*psbA-trnH*, *rpoC1, rbcL, matK*) and two noncoding nuclear ribosomal DNA (ITS, ITS2), we have got potential barcodes of plants in Fabaceae. The results suggests that (Ⅰ) ITS2, *psbA-trnH, rpoC1, rbcL* all show great universality and amplified well across all three subfamily except ITS and *matK*. (Ⅱ) ITS2 and *psbA-trnH* are two more discriminatory locus in Fabaceae. (Ⅲ) For species discrimination, ITS2 and *psbA-trnH* both work well with high levels of authentication. Overall, our findings indicates that DNA barcoding is an efficient and powerful taxonomic tool in Fabaceae. ITS2 and psbA-trnH show bright future as strong barcodes for Fabaceae in our study.

DNA BARCODING ON WOODY PLANTS IN JAPAN

YOSHIMARU, H. (1), Ohtani, M. (1), Yoshimura, K. (1), Suzuki, M. (2), Tanaka, T. (2), Noshiro, S. (1), Fujii, T. (1), Tachida, H. (3), Nagai, H. (3), Ito, M. (4), Jinbo, U. (4), Tsumura, Y. (1) and Kawahara, T. (1)

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There are more than a thousand of woody plant species listed in Japanese flora. As basic samples for DNA barcoding, we have been collecting herbarium specimens and DNA samples on these woody plant species. Expeditions for sampling have been carried out at about twenty forest stands

through south to north in Japan. At least one specimen was collected for each species which was seen in each forest stand, and therefore some number of samples within species was gained according to their distribution area. About six hundreds and fifty species with more than 3000 individuals have been collected by these sampling activities. But most of these species are common species with wide-ranged distribution area, and we should aim at rare species and species with restricted distribution area, for example, high mountains or marginal islands from now. Using about 390 species with about 1080 individulas out of these samples, DNA sequences are analyzed on rbcL and trnH-psbA as first targets of DNA barcoding. We will introduce the identification and species diversity of Japanese woody plants based on these barcoding data.

BARCODING JACKFRUITS FOR DOCUMENTATION, DOMESTICATION AND CONSERVATION IN BANGLADESH

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The fertile soil and monsoon climate of Bangladesh have been important factors in making this region a center of diversity for a wide range of plant species, including jackfruit, Artocarpus heterophyllus (Moraceae). The place of origin and wild ancestor of jackfruit is unknown , but it is believed to be indigenous to the rainforests of the Western Ghats and the Andaman Islands (India). It is cultivated at low elevations throughout Bangladesh, India, Burma, Ceylon, Southern China, Malaya and the East Indies. It is common in the Philippines, both cultivated and naturalized. It has also been introduced throughout the tropics. The jackfruit tree is monoecious, with separate staminate and pistillate inflorescences on same tree. A wide and undocumented diversity of this species has been observed throughout Bangledesh. Being a multipurpose tree, yielding food, fodder, timber and fuel, it has played an important role in the rural economy of Bangladesh. With it's Center of Origin and Center of Diversity in this region it has rightly been selected as the National Fruit of Bangladesh.

Several reports indicate that a moderate level of genetic erosion of genetic diversity has already occurred in Bangladesh. In addition to loss of jackfruit trees due to logging mature trees for timber and clearing land for agriculture, market demand for jackfruit may lead to the replacement of local diversity with uniformity of exotic genotypes and local consumption with distribution to large urban markets. As a cross pollinated and seed propagated species, the diversity in jackfruit populations result from the breeding system and natural selection associated with local environmental differences (evolution) or human selection and the preferences of the local community cultivating them (domestication). As an under-utilized crop, jackfruit has escaped intensive selection and cultivation as yet, as observed in numerous villages of Bangladesh and India. For these reasons, a wide range of variation in vegetative and reproductive characters are expected.

Poster Abstracts

Plant Working Group

The genetic diversity of jackfruit is a valuable resource for the present and future and Bangladesh is expected to be home to rich morphological and genetic variability and possibly harbor wild jackfruit. The documentation of this genetic resource is a necessary first step in understanding and conserving the diversity for long term sustainable use. The current results of the authors' research as reported here and villagers' knowledge base indicate the need in Bangladesh for (a) detailed documentation and evaluation of jackfruit fruit types, (b) descriptors for different jackfruit plant and fruit types, (c) identification of preferred jackfruit cultivars, (d) genetic assessment of jackfruit diversity, and (e) establishment of participatory on-farm conservation programs. Barcode based identification of genotypes, selection of high quality types and on-far, participatory conservation will be done in the diversity rich areas of Bangladesh and a phylogeny analysis of the species will be initiated.

MOLECULAR MARKER APPROACH IN PLANT BIODIVERSITY AND TAXONOMY STUDIES AT THE BOTANICAL GARDEN OF VILNIUS UNIVERSITY

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Large collections of plant accessions are conserved in the Gene banks and Botanical gardens around the world. However, many of these collections are not characterized well neither genetically nor phenotypically. With the recent development of genomics and bioinformatics, many international and interdisciplinary groups are brought together across the world aiming to contribute towards the knowledge of genetic background of plant diversity. Molecular markers have big advantage that they are 'neutral' and more numerous than morphological markers which allows precise estimations in genotyping.

The Botanical Garden of Vilnius University takes a part in the National Programme of Plant Genetic Resources since 1994. There are two Network sites established at the Botanical Garden of Vilnius University: Lithuanian Coordination Centre for Genetic Resources and Protection of Ornamental Plants, and the Central European data base for *Ribes* L. and *Rubus* L. genera (www.ribes-rubus.gf.vu.lt). There are about 10.000 plant accessions from 190 families and 866 genera in the collections. The oldest and most abundant collections among horticultural plants are the collections of currant and gooseberry. Nurseries are established for the conservation of the genetic resources of ornamental plants: *Iris* L., *Dahlia* Cav., *Gladiolus* L., *Paeonia* L. and others. The collection of woody plants includes more than 2500 specimens.

RAPD studies have been carried out at the Botanical Garden of Vilnius University for the assessment of genetic diversity in the collections of *Vitis vinifera* L.*, Rubus idaeus* L., *Ribes* spp. and *Lonicera* spp. Recently we have started application of plastid marker approach for discrimination between twelve *Lonicera* L. species and subspecies.

Floorplan of Poster Abstract Display Area

Index of Presenters & Coauthors

Index of Presenters & Coauthors

Index of Presenters & Coauthors

Index of Presenters & Coauthors

Index of Presenters & Coauthors

L

M

Index of Presenters & Coauthors

Index of Presenters & Coauthors

Q

R

S

Index of Presenters & Coauthors

Index of Presenters & Coauthors