**Informe final* del Proyecto GU001**
**Reunión: North and Central American Linkages for the DNA barcoding of Fish**


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| **Responsable:** | Dr. Manuel Elías Gutiérrez |
| **Institución:** | El Colegio de la Frontera Sur |
| **Dirección:** | Carretera Panamericana y Periférico Sur s/n, María Auxiliadora, San Cristóbal de Las Casas, Chis, 29290 , México |
| **Correo electrónico:** | melias@ecosur.mx |
| **Teléfono/Fax:** | Tel: 01 983 835 0440 ext. 4313 |


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**Resumen:**


Los Barcodes son una nueva técnica molecular, basada en la secuenciación de un pequeño fragmento del genoma mitocondrial denominado COI, que sirve para identificar las especies de peces y de todos los demás animales eukariotas. Entre las grandes aplicaciones prácticas de este herramienta son el reconocimiento de especies nuevas para la ciencia, desarrollo de inventarios de peces, la protección de especies en peligro de extinción, encontrar los vectores de enfermedades, plagas, especies invasoras, y todos los ámbitos en los que la reglamentación reconocimiento de la especie es crítica. Esta reunión será fundamental para establecer las estrategias y alianzas a nivel internacional para dar un impulso definitivo para la creación de la biblioteca de código de barras de las especies de peces del continente americano. GU001


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# North and Central American Linkages for the DNA Barcoding of Fish
# June 5-6, 2008

## Report of activities

## Executive summary

Mexico has the commitment to become a regional node in the International Project for the Barcode of Life (iBOL). In view to accomplish this goal, it was organized this meeting in El Colegio de la Frontera Sur (ECOSUR) at Chetumal city, Quintana Roo state, Mexico. Main goals were to give difusion of the barcoding, to help organize regional Fish-Bol participants and get them to find ways to obtain funding in addition to introduce the Consortium for the Barcode of Life (CBOL), FishBOL, and Mexican Barcode of Life (MEXBOL) initiatives, as well as the first results on fish barcoding from the region. Once established MEXBOL, iBOL compromised $1 M contribution per year in analytical and informatics support. Collections, actual projects, potential participation and fundings were explored. In total 53 researchers with interests in ichthyology participated in the meeting. Nine collections from six countries (Canada, USA, Mexico, Belize, Guatemala, and Nicaragua) were represented. In total 20 talks were presented and two round table discussions were held. Among the main results, it was presented MEXBOL proposal (still to be funded by Consejo Nacional de Ciencia y Tecnología, CONACYT), consisting in the establishment of three national labs (located at El Colegio de la Frontera Sur, Centro de Investigaciones Biológicas del Noreste and Instituto de Biología) devoted to high volume PCR (30,000 reactions). Mexico will be fully integrated to the network system established through the Barcode of Life Database (BOLD, www.boldsystems.org), and a mirror of it will be developed by Comisión Nacional Para el Uso y Conocimiento de la Biodiversidad (CONABIO). All participants were invited to know BOLD and to register in this system with a project. 13 Eppendorf plates were distributed, as well as instructions and database files, to colleagues from Mexican new participant institutions, Belize and Guatemala. They will start the exploration how the system works. All talks explained how the collections are organized, potential participation of already running collections and projects, and results on barcoding adult and larvae matching of marine and freshwater fish from North and Central America. All participant countries expressed strong intention to participate in the FISH-BOL, with an expressed desire to further participate in iBOL and the first attempts to coordinate the efforts and to explore potential fundings were established.

## Detailed outline

Organizing Comitee: Martha Valdez-Moreno (mvaldez@ecosur.mx), Manuel Elías-Gutiérrez (melias@ecosur.mx), Robert Hanner (rhanner@uoguelph.ca) and David Schindel (schindeld@si.edu)

Funding Institutions: Consortium for the Barcode of Life (CBOL), Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO, Grant GU001) and Consejo Quintanarroense de Ciencia y Tecnología.

Logistic and Technical support: El Colegio de la Frontera Sur, Chetumal Unit.

Number of countries represented: Six (Canada, USA, Mexico, Belice, Guatemala and Nicaragua)

Total number of participants: 53

## Goals for the meeting:

1) Clarifiy the concept and applications of fish barcoding

2) To invite all researchers dealing with fish diversity to participate in the Fish-Bol campaign

3) Raise awareness about the uses of DNA barcoding and potential applications among research, fisheries, aquaculture and protection of nature organizations.

4) Raise awareness to the pitfalls of barcoding, focusing on the responsible application of the technique

5) Demonstrate actual applications of DNA barcoding on different issues of fish biology, i.e. matching eggs and all immature stages with adults.

6) Search of potential funding opportunities to participate in the barcoding

Round table discussion among all attendants.

June 5, 2008

Topic: Diversity and barcodes: Highlights and weakness

Chair: Robert Hanner, University of Guelph

Notes taken by Juan Jacobo Schmitter-Soto

Questions

1) Which have been the main contributions of fish barcoding to understand their biodiversity?
2) Where some weaknesses of the barcoding have been detected?
3) Are the barcodes going to replace taxonomists?
4) Which is the importance of the vouchers and well curated collections in the barcoding?
5) Who is the owner of all data and DNA templates generated by the barcodes?

Discussion generated

- Variation in internally fertilized fish is very low
- Divergent haplotypes in same area may be overlooked species, cryptic
- African rift lake cichlids: barcodes do not work, speciation too incipient
- Species flock in Caribbean, hamlets, *Hypoplectrus*, could be similar
- There could be other usable markers, when COI shows no difference
- Barcoding is no substitute for taxonomy. It makes id easier and faster, so taxonomists can tackle other problems
- For trivial ids, barcoding is not required
- Supposed widely-distributed species can be species-complexes. Barcodes could discover this
- Barcodes underscore importance of collections and voucher specimens
- There is reciprocal illumination between barcodes and other tools
- There is no threshold of divergence that defines species. Therefore one needs vouchers
- There has never been so much info available online (e.g. Biodiversiy Heritage Library)
- It is very important to label barcoded voucher specimens appropriately
- Data are owned by the person who starts the project in BOLD
- Data are kept private until the owner says otherwise
- Other molecular markers can be put in BOLD, not just COI

- Part of the info is always public, not the sequences, but just the ids
- BOLD gets now about 3000 hits/day
- One can include cf., aff., etc.
- BOLD is still monolingual. It should be translated to Spanish and other languages
- 17% of the world fishes have already been barcoded
- In Mesoamerica, out of 7383 species 16% have been barcoded
- One can generate a list of missing taxa, a "shopping list". The Fish-BOL website is a tool to plan collecting
- The aim is to create a community. Papers and meetings can be advertised in the web, links put. (there is already one to FishBase)
- Label tissue submissions destined for the US and Canada as "diagnostic specimens". In Brazil and India, sequencing will be done in the country itself because specimens cannot be exported
- *Merluccius* from New Zealand and Argentina came out together in barcode
- There are tree-based id protocols as well as % seqence similarity scores
- Although there is no threshold defining species, 4-6% divergence is typical at species level
- BOLD trees are phenograms, formed by neighbor-joining, not cladograms. NJ is used for speed. Moreover, the aim is id, not phylogeny
- Id is a phenetic exercise
- Also, 550 bp is little for phylogeny, it becomes saturated. Moreover, it is problematic to attempt to build geographically-limited phylogenies that do not include sister taxa
- Nicaragua: there is a University, UCA, with a molecular lab. They work with Cichlidae, with 400 samples from crater lakes. They know this project. They have a sequencer. They sent a letter proposing collaboration
- The webpage (www.boldsystems.org) is the way to participate in the project. Participants in this workshop should contact Martha for coordination
- Boxes are sent out for sampling
- Balart: does FishBol include MexBol? Yes, partially (the fish portion of MexBol), but the funding is separate. MexBol is funded by CONACYT.
- Lozano-Vilano: worried about access to the data. No need to, everything rests in the hands of the owner of each project. It's their data, their samples. However, some day the data will be public
- Out of nationalism (sic!, Manuel), CONABIO will make a mirror-site
- PCR goes out to Canada. The DNA can be repatriated, if there are cryofacilities

- Vásquez-Yeomans: What if I never free up my data? If it never becomes published, it never existed. The genomics community makes their data available as soon as they are generated; barcoders don't, because of the need to validate ids. The compromise is to publish the sequences, but without the validation. Clean-up is necessary.
- In Canada, the maximum time to publish is 1 yeaar. CONABIO allows for five years, unless the project was assigned directly.

General conclusion

Barcodes are a useful tool that will aid in progress of taxonomy, not to replace it.

As an international enterprise, BOLD should be available in main spoken languages. Mexico will involve in barcoding through their main academic institutions, and vouchers will be stored in any of the main, well curated collections available.

Data and all information generated in the barcodes belong to the person that started an individual project. There should be a reasonable term to publish the data (maybe a limit of five years).

Second Round Table
June 6, 2008

Topic: Linkages and collaboration possibilites for the fish barcoding from Central to North America

Chair: Benjamin Victor,

Notes taken by Juan Jacobo Schmitter-Soto

Questions:

1) How many species of fish are there in North and Central America, and how many have been barcoded?

2) Where are there frozen or ethanol tissue collections that can be barcoded and what sources of newly collected fish can be directed into barcoding?

3) What facilities and people are ready to do barcoding, and what other groups would like to get involved?

4) What training and equipment is needed?

5) What are the most interesting taxa and areas for barcoding, and what sources of funding might be available?

6) Who will organize projects and develop grant proposals?

7) Where should the proposals be submitted for funding, and who will write and submit them?

Discussion generated

- Many freshwater collections in Mexico; marine, more Caribbean than Pacific. Approach should be different, because there are few endemics in Caribbean and many in Pacific and freshwater. 10% of the Gulf of California fishes are endemic, about 90 spp. So, it's preferable to collect in Pacific, to build a database.
- The book on common names of North American fishes lists 3700 described species, including species to 100 m deep. 1200 are freshwater, 1000 are reef-associated in the Caribbean, some 1200 Atlantic, 1200 Pacific (figures should be checked). These numbers are increasing
- It is not so important to barcode a species that has already been barcoded elsewhere, except when taxonomic problems are expected or known
- Endemism implies need for local collection. In Mexico, 140 endemics in Pacific, few if any in Atlantic, some 400 in freshwater. Islands increase endemism.
- Easier to collect in Mexico than in Colombia
- Priority: more species, not barcoded before. Make a working list, "wish-list"
- To work with mid-water and deep-sea species, we need a "champion", somebody committed to this
- People should propose their taxa of interest
- Many species have already been captured and barcoded in US waters, but Caribbean species not so much, not even in the southern Gulf of Mexico. However, many Caribbean species occur in Florida, Puerto Rico, US Virgin Islands, etc.
- Maybe, for already-barcoded species, one should capture just 1-2 specimens. There are no a priori exclusions
- IBUNAM would share material. It has a lot of myctophids
- Focus should be on missing species, but also doubtful ones. Check in BOLD, ask colleagues
- Some workers have frozen/ethanol tissues, but no established collections. Need to coordinate. CIBNOR is also a node. In order not to duplicate efforts, check BOLD and institutional databases; privilege can be given to allow people to check what we are working on
- Every node is contacting nearby collections; regionalize
- Books with collection descriptions, already consulted
- There will be a moderate geographic overlap
- But, the wish-list for Mesoamerica would have 5000 species, and it doesn't tell who is working what. However, one can map, check where the samples came from
- With analyze-only, one can see the info, maybe not the tree. That privilege can be given to this group
- Merge-project needed? No, only virtual
- This can be done allowing the public user to edit specimens
- Know BOLD! Search in Google, "barcode of life"
- Guatemala already recommended the Universidad del Valle de Guatemala. Nicaragua: UCA. Belize: will work with ECOSUR and Smithsonian. Luis Sierra, Costa Rica,

interested. Wil Matamoros, Honduras, couldn't come, but interested too. Birmingham, Panama.

- Open blog about sampling campaigns in BOLD? Who will keep it updated? Better to do that regionally. Martha will.
- Training needed. Instructions for filling boxes, to be sent by e-mail. If needed, Martha will travel to train people
- An intention letter was presented by Nicaraguan representative, Alejandro Cotto.
- BOLD gives a process-id associated to catalog number
- Funding? CONACYT promised $50M pesos in 5 years (all groups). $10M first year, to establish nodes. $10M second year, for reagents and cryopreservation. Send projects to the committee. CONABIO will participate with fundings still to be established. This year the latter mention that barcoding will be among his priorities and commited to create a mirror for BOLD in relation to Mexican databases. They will make a link to their databases in REMIB (see www.conabio.gob.mx) for specimens. The mode of participation will be trough calls and submitted projects.
- In reply to this commitment from CONACYT and CONABIO, three Mexican institutions El Colegio de la Frontera Sur (ECOSUR), Centro de Investigaciones Biológicas del Noreste (CIBNOR) and Instituto de Biología from Universidad Nacional Autónoma de México (IBUNAM) prepared an Initiative for the Mexican Project for the barcode of Life (MEXBOL) that was submitted to CONACYT last February, to be evaluated by CONACYT for funding.
- If MEXBOL gets approved, there will be funding for ECOSUR, IBUNAM, CIBNOR to prepare three labs for high volume DNA extraction and pcr (30,000 reactions/year/lab). Sequencing will be carried in Canada. Every person leading the node will make his own calls for potential participants. Representatives will be: ECOSUR, Manuel Elías-Gutiérrez (melias@ecosur.mx) ; CIBNOR, Sergio Ticul Alvarez Castañeda (sticul@cibnor.mx); IBUNAM, Patricia Escalante Pliego (tilmatura@ibiologia.unam.mx) Central America will designate her leaders.
- MEXBOL will open a website (by CONABIO)
- Mexico will not finance Central America, but stipendia could be given to students. UCA has funds for field work; for marine work, Instituto de Pesca. Only guidance is required. To guarantee a correct job, we will all observe ourselves.
- Check European Unions grants
- Consejos de Ciencia de Centroamérica have interchange with CONACYT; an agenda is being integrated. Everardo Barba leads
- Investment needs not be large
- NOAA has supported
- There are other committees, not just fishes
- Send e-mail to everybody attending this mtg. Send contact info to Bob. Put in FishBol
- www.biorepositories.org, to register collections, to make them acknowledged in GenBank, etc.
- A result of this workshop: people in the group, participant collections. Define how many specimens will be processed. It takes two full days to fill a box and the data
- Make our powerpoint presentations public on the site? Yes, also contact info and abstracts
- Meetings about other taxa? There was funding available for this one, but other meetings could be useful. Actual strategy: to present about barcoding and goals in scheduled

meetings , e.g. the Congreso de Zoología by the end of August, The VIII International Symposium on Cladocera by the end of October.

General conclusions

North American fish lists approximately 3700 described species, including species to 100 m deep. 1200 are freshwater, 1000 are reef-associated in the Caribbean, some 1200 Atlantic, 1200 Pacific. These numbers are increasing. From these numbers, endemics in Mexico range to 540 in round numbers.

Real challenge will be to collect deep fish in both seas.

There should be coordination for not to repeat efforts in barcoding, the best tool is the FISH-BOL web site which can be used to generate lists of taxa and the number of specimens barcoded for each FAO region.

There is a need to familiarize potential participants with BOLD. Some Eppendorf plates were given to people to start with their first barcoding project. Once really involved, the people will send an e-mail to Robert Hanner (rhanner@uoguelph.ca), to be included in the list for FishBold.

Main funding source will be CONACYT and CONABIO in Mexico, but there are other potential sources for fundings. Seed will be the money granted by CONACYT to establish three national labs with regional influence or group experience. Three Mexican institutions El Colegio de la Frontera Sur (ECOSUR), Centro de Investigaciones Biológicas del Noreste (CIBNOR) and Instituto de Biología from Universidad Nacional Autónoma de México (IBUNAM) prepared an Initiative for the Mexican Project for the barcode of Life (MEXBOL). If this initiative is approved, a comitee established with the representatives will establish and make public calls to invite other institutions to participate in MEXBOL (Mexican Barcode of Life). Any further question can be directed to the representatives of the three labs: ECOSUR, Manuel Elías-Gutiérrez (melias@ecosur.mx) ; CIBNOR, Sergio Ticul Alvarez Castañeda (sticul@cibnor.mx); IBUNAM, Patricia Escalante Pliego

CONABIO will prepare a mirror for BOLD and links to their databases already published in REMIB.

All participant countries are interested, Everardo Barba will be a lead to establish possible fundings in collaborate work with Central America. He already sent a first communication, previous to this report. Alejandro Cotto from Nicaragua presented a serious intention letter to participate in iBOL.

Training will be available through information published and if necessary in regional workshops.

It is necessary to register main collections from Mexico in biorepositories, and try to get as many as possible data online. Part of this task is already done through the database REMIB from CONABIO and the UNIBIO, the database for the collections from IBUNAM (www.ibiologia.unam.mx).

## Program

Thursday, June 5

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| 08:15-08:30 | Transportation to ECOSUR |
| 08:30-09:30 | Registration of participants |
| 09:30-09:45 | Welcome to Fish-Bol Meeting (Alberto de Jesús Navarrete, Director of the unit) |
| 9:45-10:15 | The barcode of life: general remarks and the Mexican commitment (Manuel Elías Gutiérrez) |
| 10:15-10:45 | The Fish Barcode of Life Initiative (Robert Hanner) |
| 10:45-11:00 | Coffee Break |
| 11:00-11:30 | The Barcode in the National Collection of Fish of the IBUNAM (Héctor Espinosa Pérez) |
| 11:30-12:00 | Analyses and perspectives of the Ichtyological Collection at the Facultad de Ciencias Biológicas from the Universidad de Nuevo León (Lourdes Lozano Vilano) |
| 12:00-12:30 | The ichthyological collection at CIBNOR and its potential for the FISH-BOL project in the northwest of Mexico (Eduardo Balart) |
| 12:30-13:00 | Coastal Fish studies at north of Baja California, Mexico: a 16 years history (Jorge Rosales Casián) |
| | |
| 13:00-15:00 | Lunch |
| | |
| 15:00-15:30 | Barcoding of the marine fish of California (Phil Hastings) |
| 15:30-16:00 | The fish collection at CIIDIR, Oaxaca (Emilio Martínez Ramírez) |
| 16:00-16:30 | The fish collection (ECOSC) from Chiapas (Rocio Rodiles) |
| 16:30-17:00 | The fish collection at ECOSUR Chetumal (Jacobo Schmitter) |
| 17:00-17:15 | Coffee Break |
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| 17:15-19:00 | Round table: Diversity and barcodes: Highlights and weakness. Chair: Bob Hanner |

Friday, June 6

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|---|---|
| 08:15-08:30 | Transportation to ECOSUR |
| 08:30-09:30 | Integrating ichthyology from Yucatan Peninsula (María Eugenia Vega) |
| 09:30-10:00 | Freshwater fishes and water status in Mexico (Salvador Contreras) |
| 10:00-10:30 | DNA barcoding and north American freshwater fish diversity (Rick Mayden) |
| 10:30-11:00 | Coffee Break |
| 11:00-11:30 | Advances in Mexican fish barcoding: freshwater and marine (Martha Valdez) |
| 11:30-12:00 | The collection of fish larvae in ECOSUR and the barcoding (Lourdes Vásquez Yeomans) |
| 12:00-12:30 | Barcoding reef fish larvae (Benjamin Victor) |
| 12:30-13:00 | Inventory of marine species and current status of collections of fish in Nicaragua (Alejandro Cotto) |
| | |
| 13:00-15:00 | Lunch |
| | |
| 15:00-15:30 | Ichtyology in Guatemala (Rolando Wer) |
| 15:30-16:00 | Identifying Black Grouper (Mycteroperca bonaci) spawning aggregations of Belize (Mito Paz) |
| 16:00-16:30 | FishBold Canada-Progress and current projects (Dirk Steinke, Videoconference) |
| 16:30-17:00 | Cofee Break |
| 17:15-19:00 | Round table: Linkages and collaboration possibilites for the fish barcoding from Central to North America Chair: Benjamin Victor |

# FISH-BOL June 5-6
# Participants

| Name | Affiliation | Contry | E-mail |
|---|---|---|---|
| Aguirre Villaseñor Hugo | Instituto Nacional de la Pesca | Mexico | Haguirre@ola.icmyl.unam.mx |
| Arena Ortíz Maria Leticia | UMIDI-SISAL UNAM | Mexico | mlao@hp.fciencias.unam.mx |
| Balart Páez Eduardo | CIBNOR La Paz | Mexico | ebalart04@cibnor.mx |
| Barba Macías Everardo | ECOSUR-Villahermosa | Mexico | ebarba@ecosur.mx |
| Campos Dávila Lucia | CIBNOR La Paz | Mexico | lcampos04@cibnor.mx. |
| Cázarez Carrillo Dalia | ECOSUR-Chetumal | Mexico | stella_cazarez@hotmail.com |
| Contreras Balderas Armando | Universiad Autónoma de Nuevo León | Mexico | arcontre@fcb.uanl.mx |
| Contreras Balderas Salvador | Bioconservación AC, Nuevo León | Mexico | saconbal@gmail.com |
| Cohuo Colli José Angel | ECOSUR-Chetumal | Mexico | jacc85@hotmail.com |
| Cotto Sánchez Alejandro | Mede Pesca | Nicaragua | ictiosp@yahoo.es |
| Dzul Panti Angel Manuel | Cinvestav IPN Unidad Mérida Yucatán | Mexico | adzul@mda.cinvestav.mx |
| Espinosa Pérez Héctor | Universidad Nacional Autónoma de México | Mexico | hector@servidor.unam.mx |
| Elías Gutiérrez Manuel | ECOSUR-Chetumal | Mexico | melias@ecosur.mx |
| Escobedo Cabrera Enrique | ECOSUR-Chetumal | Mexico | escobedo@ecosur.mx |
| Espinoza Sosa Engrih | ECOSUR-Chetumal | Mexico | |
| García Morales Alma | CONABIO | Mexico | almarotifer@yahoo.com.mx |
| García Rodríguez Francisco | CICIMAR | Mexico | fj_gr@yahoo.cpm |
| Guerrero Zárate Rocío | Cinvestav IPN Unidad Mérida Yucatán | Mexico | rguerrero@mda.cinvestav.mx |
| González Garza Idalia | Cinvestav IPN Unidad Mérida Yucatán | Mexico | bigzzg@gmail.com |
| González Paredes Wuiliam | EPOMEX | Mexico | wuilliam_gon@hotmail.com |
| Hanner Robert | Biodiversity Institute of Ontario, University of Guelph | Canada | rhanner@uoguelph.ca |
| Hastings Phil | Scripps Institute of Oceanography | USA | phastings@ucsd.edu |
| Heredia Hernández Angeles | CINVESTAV-Mérida | Mexico | gelos_mda@hotmail.com |
| Hernández Armas Lidia | Universidad Autónoma Metropolitana - Xochimilco | Mexico | lha93@yahoo.com.mx |
| Hernández Ruiz Hortencia | UAM-Xochimilco | Mexico | hortencia0702@hotmail.com |
| Lozano Vilano Lourdes | Universiad Autónoma de Nuevo León | Mexico | marlozan@fcb.uanl.mx |
| Lizama Uc Gabriel | Universidad Nacional Autónoma de México | Mexico | glu@hp.fciencias.unam.mx |
| Landero Figueroa Marcela | Cinvestav IPN Unidad Mérida Yucatán | Mexico | mlandero@mda.cinvestav.mx |
| Martin Tun Mario | Cinvestav IPN Unidad Mérida Yucatán | Mexico | mmartin@mda.cinvestav.mx |
| Martínez Ramírez Emilio | CIIDIR Oaxaca | Mexico | emmartinez@ipn.mx |
| Mayden Richard | Saint Louis University | USA | maydenrl@slu.edu |
| Medina Quijano Humberto | Cinvestav IPN Unidad Mérida Yucatán | Mexico | hmedina@mda.cinvestav.mx |
| Nourisson Coralie | ECOSUR-Chetumal | Mexico | Coralie.nourisson@gmail.com |
| Ortíz León Héctor | Instituto Tecnológico de Chetumal | Mexico | oilh@yahoo.com.mx |
| Pimentel Camarillo Melissa | Instituto Tecnológico de Chetumal | Mexico | melissapimentelc@hotmail.com |
| Mito Pérez Guillermo | Green Reef Environmental Institute | Belize | greenreef@gmail.com |
| Prado Cuellar Blanca | ECOSUR-Chetumal | Mexico | bprado@posgrado.ecosur.mx |
| Ramírez Eloy | Instituto Tecnológico de Chetumal | Mexico | eloy130811@hotmail.com |
| Reyes Avalos Aldo | Instituto Tecnológico de Chetumal | Mexico | tortu_spider@hotmail.com |
| Guadalupe Rincón | ECOSUR-Chetumal | Mexico | evenstar_890@hotmail.com |
| Rincón Sandoval Alberto | Cinvestav IPN Unidad Mérida Yucatán | Mexico | arincon@mda.cinvestav.mx |
| Rodiles Hernández Rocío | ECOSUR San Cristóbal | Mexico | rrodiles@ecosur.mx |
| Rodríguez Gutiérrez Martha | UAM-Xochimilco | Mexico | rogm0211@crreo.xoc.uam.mx |
| Rosales Casián Jorge | CICESE | Mexico | jrosales@cicese.mx |
| Schmitter Soto Juan Jacobo | ECOSUR-Chetumal | Mexico | jschmit@ecosur.mx |
| Sosa Vásquez Carolina | ECOSUR-Chetumal | Mexico | evenstar_890@hotmail.com |
| Steinke Dirk | Biodiversity Institute of Ontario, University of Guelph | Canada | dsteinke@uoguelph.ca |
| Terán Juárez Sergio A. | Instituto Tecnológico de Chetumal | Mexico | bioente_tejuse@hotmail.com |
| Valdez Moreno Martha | ECOSUR-Chetumal | Mexico | mvaldez@ecosur.mx |
| Vásquez Yeomans Lourdes | ECOSUR-Chetumal | Mexico | luluvaye@yahoo.com.mx |
| Vega Maria Eugenia | CINVESTAV Mérida | Mexico | maruvega@mda.cinvestav.mx |
| Victor Benjamin | Ocean Science Foundation | USA | ben@coralreeffish.com |
| Wer Rolando | Investigador colaborador de CONAP | Guatemala | exoticdr@hotmail.com |

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# North and Central American Linkages for the DNA Barcoding of Fish

**June 5-6, 2008**

**El Colegio de la Frontera Sur**

**Chetumal, Quintana Roo,**

**Mexico.**

## About the Speakers

**Name: Eduardo Francisco Balart Páez**

**Institution:** Centro de Investigaciones Biológicas del Noroeste, S.C.
**Postal Address:** Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23090.
**Phone/FAX**: (+52)(612)1238436 / (+52)(612)1253633
**E-mail:** ebalart04@cibnor.mx
**Current position**: Investigador Titular "A"; Curador de la Colección Ictiológica del CIBNOR.
**Primary research or administrative involvement:** Taxonomy, systematics, and ecology of fishes.
**Groups of special taxonomic interest:** Marine and freshwater fishes; in second place Crustacea, Echinodermata, and other macroinvertebrates associated to reefs.

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**Name: Salvador Contreras Balderas**

**Institution:** Universidad Autónoma de Nuevo León
**Postal Address:** Apdo. Postal 504, San Nicolas, N. L., 66450 Mex
**Phone/FAX:** (52-81) 8313-1641
**E-mail:** saconbal@prodigy.net.mx
**Current position:** Emeritus Professor
**Primary research or administrative involvement:** Frewhwater fish.
**Groups of special taxonomic interest:** Ostariophysi, Atherinomorpha, Cichlidae.

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**Name: Alejandro Cotto Sánchez**

**Institution:** Food and Agriculture Organization (FAO)
**Postal Address:** 1524 Managua
**Phone/FAX:** (505) 2760425-26 Fax: 2551193
**E-mail:** ictiosp@yahoo.es alejandro.cotto@fao.org.ni
**Current position:** Fisheries and Aquaculture Coordinator
**Primary research or administrative involvement:** Development projects
**Groups of special taxonomic interest:** Lutjanidae, Sciaenidae, Centropomidae, Carangidae, Haemulidae, Serranidae.

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**Name: Manuel Elías Gutiérrez**

**Institution:** El Colegio de la Frontera Sur-Chetumal
**Postal Address:** Av. Centenario Km 5.5 Chetumal, Quintana Roo, Mexico 77014
**Phone/FAX:** (+52) 98350440 ext. 4313
**E-mail:** melias@ecosur.mx
**Current position:** Research
**Primary research or administrative involvement:** Taxonomy and Systematics of freshwater zooplancton.
**Groups of special taxonomic interest:** Cladoceran, Copepoda and Rotifera.

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**Name: Héctor Espinosa Pérez**
**Institution:** Departamento de Zoología, Instituto de Biología, UNAM
**Postal Address:** Cd. Universitaria, 3er Cto. Extrior S/N. A.P. 70-153 Mexico 04510 D.F.
**Phone/FAX:** 0155 5500164
**E-mail:** hector@servidor.unam.mx
**Current position:** Ichthyology Curator
**Primary research or administrative involvement:** México fish diversity
**Groups of special taxonomic interest:** Marine, brackish and freshwater fishes

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**Name: Robert Hanner**

**Institution:** University of Guelph
**Postal Address:** 50 Stone Rd East
**Phone/FAX:** 519.824.4120 x53479
**E-mail:** rhanner@uoguelph.ca
**Current position:** Associate Director, Canadian Barcode of Life Network
**Primary research or administrative involvement:** Campaign Coordinator for FISH-BOL
**Groups of special taxonomic interest:** Fish

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**Name: Phillip A. Hastings**

**Institution:** Scripps Institution of Oceanography, University of California San Diego
**Postal Address:** 9500 Gilman Drive La Jolla CA 92093-0208
**Phone/FAX:** (858) 822-2913; (858) 822-3310(fax)
**E-mail:** phastings@ucsd.edu
**Current position:** Profesor and Curator of Marine Vertebrates
**Primary research or administrative involvement:**
**Groups of special taxonomic interest:**

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**Name: Emilio Martínez Ramírez.**

**Institution:** Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca del Instituto Politécnico Nacional (CIIDIR OAXACA IPN).
**Postal Address:** Calle Hornos Nº 1003, Municipio Santa Cruz Xoxocotlán, C. P. 71230. Ap. Post. 674 (Ofna. Central), Oaxaca, México.
**Phone/FAX:** (52) (951) 5170400, 5170610 y 5171199. Ext. 82750. Tel. red IPN (52) (55) 57296000.
**E-mail:** emmartinez@ipn.mx, emartinr@hotmail.com
**Current position:** Profesor-investigador.
**Primary research or administrative involvement:** Ichthyology of Oaxaca.
**Groups of special taxonomic interest:** Freshwter fishes.

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**Name: Richard Mayden**

**Institution:** Departament of Biology, Saint Louis University
**Postal Address:** 3507 Laclede Ave, Department of Biology, Saint Louis University, St. Louis, Missouri, 63103 USA
**Phone/FAX:** 001-314-977-3658
**E-mail:** cypriniformes@gmail.com
**Current position:** Professor, Endowed Chair
**Primary research or administrative involvement:** Coordinator for North American Freshwater Fishes Barcoding Chairman, Department of Biology, Saint Louis University Endowed Chair of Research, Saint Louis University Systematics, Evolution and Biogeography of freshwater fishes
**Groups of special taxonomic interest:** Petromyzontiformes, Acipenseriformes, Cypriniformes, Characiformes, Siluriformes, Profundulidae, Fundulidae, Percomorpha

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**Name: Guillermo Mito Paz**

**Institution:** Green Reef Environmental Institute
**Postal Address:** 100 Coconut Drive, San Pedro Town, Ambergris Caye, Belize
**Phone/FAX:** (501) 226-2833/(501) 226-2766
**E-mail:**greenreef@gmail.com
**Current position:** executive director
**Primary research or administrative involvement:** Grouper Spawning Aggregation
**Groups of special taxonomic interest:** Serranids

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**Name: Rocío Rodiles Hernández**

**Institution:** EL Colegio de la Frontera Sur- San Cristóbal, Chiapas
**Postal Address:** Carretera panamericana y periferico sur s/n, 29290 San Cristobal de las Casas, Chiapas, Mexico
**Phone/FAX:** 9670-674-9000 EXT. 1322, 1325
**E-mail:** rrodiles@ecosur.mx or mariar56@mail utexas.edu
**Current position:** Research
**Primary research or administrative involvement:** Ichthyology (Ecology and Taxonomy).
**Groups of special taxonomic interest:** Lacantuniidae, Ictaluridae, Guerreidae, Heptapteridae, Ariidae, Cichlidae, Poecilidae, Characidae.

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**Name: Jorge A. Rosales Casián**

**Institution:** Centro de Investigación Científica y de Educación Superior de Ensenada, B.C. (CICESE).
**Postal Address:** Km 107 Carretera Tijuana-Ensenada, Ensenada, B.C., México
**Phone/FAX**: (646) 175-05-44; 175-05-00/ (646) 175-05-45
**E-mail:** jrosales@cicese.mx
**Current position:** Investigador Titular B
**Primary research:** Ecology and Biology
**Groups of special taxonomic interest:** Coastal Fishes

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**Name: Juan Jacobo Schmitter Soto**

**Institution:** El Colegio de la Frontera Sur
**Postal Address:** A.P. 424, 77000 Chetumal, Q.R., Mexico
**Phone/FAX:** +52-983-8350440 ext. 4302
**E-mail:** jschmitt@ecosur.mx
**Current position:** Senior Researcher
**Primary research or administrative involvement:** Systematics and Ecology of Fishes
**Groups of special taxonomic interest:** Fishes

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**Name: Dirk Steinke**

**Institution:** Biodiversity Institute of Ontario
**Postal Address:** University of Guelph, Guelph, ON, N1G2W1
**Phone/FAX:** +1-519-824-4120 x53759 +1-519-824-5703
**E-mail:** dsteinke@uoguelph.ca
**Current position:** Lead Scientist Marine Barcoding of Life (MarBOL)
**Primary research or administrative involvement:** FishBOL & MarBOL
**Groups of special taxonomic interest:** Marine: Labridae, Pomacentridae. Freshwater: Cichlidae, Poeciliidae

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**Name: Martha Elena Valdez Moreno**

**Institution:** El Colegio de la Frontera Sur
**Postal Address:** Av. Centenario Km 5.5 Chetumal, Quintana Roo, Mexico cp. 77014
**Phone/FAX:** (+52) 98350440 ext. 4307
**E-mail:** mvaldez@ecosur.mx
**Current position:** Research
**Primary research or administrative involvement:** Fish Taxonomy in particular with osteology and barcodes.
**Groups of special taxonomic interest:** Marine and freshwater fishes.

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**Name: Lourdes Vásquez Yeomans**

**Institution:** El Colegio de la Frontera Sur
**Postal Address:** Centenario Km 5.5 Chetumal, Q. Roo
**Phone/FAX:** (+52) 983 8350440
**E-mail:** luluvaye@yahoo.com.mx
**Current position:** Investigador Asociado
**Primary research or administrative involvement:** Fish Taxonomy and Ecology
**Groups of special taxonomic interest:** Marine fish larvae

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**Name: Ma. Eugenia Vega Cendejas**

**Institution:** Centro de Investigación y de Estudios Avanzados del IPN.
**Postal Address:** Km. 6 Antig. Carretera a Progreso.
**Phone/FAX:** (999)9-42-94-71
**E-mail:** maruvega@mda.cinvestav.mx
**Current position:** Research
**Primary research or administrative involvement:** Fish Taxonomy and Ecology
**Groups of special taxonomic interest:** Fishes

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**Name: Benjamin Victor**

**Institution:** Ocean Science Foundation
**Postal Address:** 4051 Glenwood St. Irvine, CA 92604
**Phone/FAX:** 949-246-2806
**E-mail:** ben@coralreeffish.com
**Current position:** Researcher
**Primary research or administrative involvement:** barcodes of reef fishes
**Groups of special taxonomic interest:** Fish: gobies, labrids, lutjanids

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**Name: Ma de Lourdes Lozano Vilano**

**Institution:** Universidad Autónoma de Nuevo León
**Postal Address:** Ap. Postal 425, San Nicolás de los Garza, Nuevo León, México, 66450
**Phone/FAX:** 52 (81)8376-2231
**E-mail:** marlozan@fcb.uanl.mx
**Current position:** Maestro de Tiempo completo y exclusivo UANL, titular B.
**Primary research or administrative involvement:** Responsable del Laboratorio y Colección de Ictiología, Facultad de Ciencias Biológicas, UANL.
**Groups of special taxonomic interest:** Cyprinodontidae, Fundulidae, Cyprinidae, Percidae, Characidae.

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**Name: Rolando Wer Aguilar.**

**Institution:** CONAP / Fundaparque Guatemala.
**Postal Address:** 9 ave. 15-51 Zona 10.
**Phone/FAX:** (502) 23328324
**E-mail:** dr4wild@yahoo.com
**Current position:** Advisor.
**Primary research or administrative involvement:** Native Fish research.
**Groups of special taxonomic interest:** Poecilidae, Cichlidae, Ariidae.

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**Name: Ana Martha Zetino Calderon**

**Institution:** University of El Salvador
**Postal Address:** Colonia Zacamil Edificio 17 No.27. Mejicanos
**Phone/FAX:** (503) 22262072
**E-mail:**marzeti@hotmail.com
**Current position:** Professor and researcher at the Biology School
**Primary research or administrative involvement:** conservation of native aquatic species in El Salvador
**Groups of special taxonomic interest:** Fishes

## Abstracts

### The barcode of life: general remarks and the Mexican commitment
**Manuel Elías Gutiérrez**

For more than 20 years mitochondrial sequence diversity has been used to distinguish closely allied species. However, 'DNA barcoding', the survey of sequence diversity in a 650 bp segment of the mitochondrial DNA gene cytochrome *c* oxidase subunit 1 (COI) has been proposed since 2003 by Hebert et al. as a standard tool for species-level identifications of all animals. Aside from the benefits of creating a DNA-based identification system, DNA barcoding is an effective tool for gaining an initial sense of the patterning of genetic divergences. Because of this fact, varied authors have suggested that DNA barcoding will aid rapid progress in traditional taxonomic work by speeding in the discovery of new species, and the recognition of synonymies. With the time barcodes will be a useful tool for all non-taxonomists to identify with confidence their specimens. Although barcoding remains controversial in some circles, a big project involving 26 nations has been launched to barcode 0.5 M species in the next five years. Mexico will play a role as Regional Node within this project, through an initiative named MEXBOL. The commitent here is to create three facilities, geographically distributed in the south (ECOSUR-Chetumal), center (Instituto de Biología, UNAM) and north (CIBNOR-La Paz) of this country. The three labs will be designed for high volume extraction of DNA and PCR. They will serve all people interested, including Central American colleagues. Sequencing will be carried in the facility at University of Guelph (Canada). In case of Mexico, funds will be provided by CONACYT (50 M Mexican pesos in the next five years), CONABIO (a mirror for BOLD) and the institutions involved (space for the labs and human resources). FishBol and CBOL will play a key role in the development of the barcoding effort here.

### The Fish Barcode of Live Initiative
**Robert Hanner**

The Fish Barcode of Life initiative (www.FISHBOL.org) is an international research collaboration dedicated to assembling a standardized reference sequence library for all fishes. New primer cocktails have been developed targeting 650 bp of the 5' end (e.g. the "Folmer" region) of the mitochondrial cytochrome *c* oxidase I (COI) gene enabling broad amplification of fishes. The resulting sequence profiles or "DNA barcodes" conform to standards established by the Consortium for the Barcode of Life (CBOL) and are typically derived from expert-identified voucher specimens archived in accessible reference collections. The Catalog of Fishes, FishBase and ITIS contribute to a taxonomic authority file for the initiative, while FISH-BOL serves as a conduit of communication for ichthyologists to provide information updates back to the aforementioned community resources. More than 4500 species have already been barcoded, with an average of five specimens per species. Current results indicate that barcodes separate >98% of previously described fish species that have so far been analyzed. Several genetically divergent specimens have also been confirmed by integrative taxonomic analysis as new species. The benefits of barcoding fishes include the global disambiguation and reconciliation of names, highlighting cases of range expansion for known species, flagging previously overlooked species and, enabling identifications where traditional methods cannot be applied. In this respect, DNA

barcoding democratizes access to species level identifications: unknown sequences, from any fish or its fillets, fins, eggs and larvae can be matched against these reference sequences using BOLD: the Barcode of Life Data System (http://www.barcodinglife.org).

**The BARCODE in the CNP of the IBUNAM**
**Héctor Espinosa pérez**

More than 2,700 species constitutes a high number, the fishes are the most abundant vertebrates in the earth and they are also it in our country. In the National Fish Collection (CNP) have been documented 259 fish Families in Mexico of the total 515 exist in the world, this is little more than 50% of the total, included the Mexican new Family Lacantunidae. In the country are known more than 310 fresh water exclusive species and 506 marine species have been documented that penetrate to the continental waters as coastal lagoons and rivers. The marine fish of two Oceans and the gulfs of Mexico and California conform one of the most diverse faunas in the world. There are more than 543 commercial species subject to use, and a list of 184 species considered in the NOM-059-ECOL 2002 in some situation of risk. The goal of the CNP is to know the diversity of fish in the country. By means of the BARCODE project we hope know the Mexican species, as well document and research about systematic and biogeography aspects of the ichthyofauna. At the moment there are more than 700 samples of freshwater, brackish and marine species to be analyzed in the project.

**Análisis y perspectivas de la colección ictiologica de la Facultad de Ciencias Biológicas de la Universidad Autónoma de Nuevo León, México**
**Lourdes Lozano Vilano**

La Colección Ictiólogica de la Facultad de la Facultad de Ciencias Biológicas de la UANL, fue iniciada en 1958 por el Dr. Salvador Contreras Balderas y entregada a la UANL en 1965. Actualmente la colección cuenta con mas de 1,000,000 ejemplares, enteros y preservados en Alcohol isopropílico al 50%, otros fijados en Alcohol etílico para estudios genéticos, igualmente esqueletizados y aclarados y teñidos; actualmente se cuenta con cerca de 18,600 lotes, pertenecientes a 134 familias, 411 géneros y 1112 especies, dentro de los cuales se tiene Material Tipo, 10 Holotipos y 32 Paratipos; además se tienen cerca de 300,000 ejemplares por catalogar, el material se ha obtenido por colectas hechas en agua dulce en todos los estados de la República Mexicana, igualmente marinos hechas en el Golfo de México, Caribe, Mar de Cortez, y Pacífico; Además contamos con material colectado y por intercambio de Estados Unidos (Texas, Arizona, Alabama, Nuevo México, Nueva York, Oregon, Florida, Louisiana, California y Oklahoma), Canadá, Guatemala y Belice, y por intercambio con diferentes museos se tienen ejemplares de Ecuador, Nicaragua, Panamá, Honduras, Costa Rica, Venezuela, Brasil, Perú, Italia, Alemania, Túnez, Algeria, Gran Bretaña, Rumanía, Sahara Central.

**The Ichthyological Collection of the CIBNOR and its potential for the Fish-Bol proyect in the northwest of Mexico.**
**Eduardo Balart**

The Ichthyological Collection of Centro de Investigaciones Biológicas del Noroeste was born in 1998. Actually the collection holds 420 species in 233 genera belonging to 103 families. One of the best represented groups in the collection are the soft bottom fishes of the west coast of Baja California Sur. The fishes of the Gulf of California are recently incorporated in the collection too, through collections from the lagoons, mangroves, and continental shelf from Baja California Sur and Sonora. However, the most interesting samples from the Gulf of California are those of cryptic and/or cryptobenthic reef fishes; many of them are endemic species and they are badly represented in most collections. Other places documented in the collection are the shallow coast of Michoacan-Guerrero. Small collections of freshwater fishes from the peninsula of Baja California, Sinaloa, San Luis Potosi, and Michoacan-Guerrero are present also in the collection. Most of the material was originally fixed in formaldehyde 10% and stored in isopropyl alcohol 50%. However recently we start to keep entire fish and/or fish tissue in ethanol for DNA studies and otoliths. Due to our location in the northwest of Mexico and to the decisive support of the actual Director of the Center, we think our collection may play an important role in the Fish-bold project. First, we have already some material in the collection to start to work on it. Secondly, with the proper support we can easily develop a sampling program for the cryptic and elusive especies of the rocky reefs and other habitats. Through the research vessel of our Center, BIP XII, we can develop a thorough plan of collection in strategic areas of the Pacific and Gulf of California. We have also the support of our branchs in Guaymas, Guerrero Negro, and Hermosillo to base collection work for the freshwater fishes of most part of the northwest of Mexico.

**Coastal Fish Studies at North of Baja California, México: A 16 years history.**
**Jorge A. Rosales Casián**

The study of coastal fishes began in 1986-1987, but formally in 1992, both at Bahía de Todos Santos, Ensenada, Baja California, México. In this last study, the fishes were monthly collected in the Todos Santos Bay-Punta Banda Estuary system to determine the fish species assemblage, their abundances, the species importance and the movements between both sites. To collect the fishes, the beamtrawl and otter-trawl (5 & 10m-depth), beach seine (<3.5 m-depth), experimental gillnet (10m-depth) and the hook-and-line (5-10m-depth) were used with replications. Following to south, next study was at Bahía de San Quintín and its adjacent coast, monthly in 1994, and by seasons up to 1999. Other sites were Punta Cabras (Ejido Erendira), Bahía El Rosario, Bahía Colonet, and recently Bahía Santa Rosalillita and Bahía de Los Ángeles (2005-2007), this last in the Gulf of California. As part of the studies, the commercial fishes as California halibut, kelp bass, barred sand bass, spotted bass, California corbina, yellowfin croaker, and spotfin croaker were separated for a major work. Species like California halibut were studied in their feeding habits, age and growth, and parasitism. Other sources that supplied fish information were the Ensenada seafood market that sale the fish catch from the Pacific and Gulf of California, the sportfishing activities and the artisanal coastal fishing.

**Barcoding of the marine fish of California**
**Philip Hastings**

With funding from California Sea Grant, this project made a concerted effort to collect and archive tissues, voucher specimens and mitochondrial sequence data for all marine fish species recorded from California waters. Sequence data were initially collected for two mitochondrial markers (cytochrome b and 16S ribosomal DNA) and later for cytochrome oxidase I (COI) with the kind assistance of FishBOL. All vouchers and tissue samples were deposited in the SIO Marine Vertebrate Collection and sequence data for approximately 500 species have been deposited with GenBank and the Barcode of Fishes Project. These data have been used for identification of fish larvae collected off southern California and stomach contents of predatory fishes from the California Current. Issues concerning the archiving of tissues and voucher specimens will be discussed.

**La Colección de Peces del CIIDIR OAXACA IPN.**
**Emilio Martínez Ramírez**

Desde que inicio sus actividades este centro de investigación, el Área de Acuacultura han realizado varias investigaciones ictiológicas, obteniendo como uno de sus resultados la Colección de Peces Continentales, con registro N$^{o}$ OAX-PEC-122-0302 de la SEMARNAP, la cual cuenta con un total de 1 795 registros curatoriales georreferenciados y 32 237 ejemplares de 153 especies correspondientes a 90 géneros y 45 familias. De acuerdo al número de registros, las familias más representadas son: Poeciliidae (531), Cichlidae (258), Characidae (129), Pimelodidae (92), Mugilidae (90), Profundulidae (88), Gerreidae (81), Gobiidae (67), Eleotridae (61), Cyprinidae (54), Centropomidae (43), Synbranchidae (44), Engraulidae (46) y Carangidae (29). Se tienen muestras de peces de los siguientes estados ordenados descendentemente de acuerdo al número de registros: Oaxaca, Guerrero, Morelos, Veracruz, Puebla, Chiapas y de México; las cuales provienen de colectas con técnicas de electropesca, pesca artesanal (atarraya, chichorro y red agallera) y comercial (barco camaronero) y donaciones. Se tienen cuatro bases de datos de los registros de la colección, los parámetros abióticos y bióticos y de la ictiofauna de la Reserva de la Biosfera de Tehuacán-Cuicatlán y del sistema lagunar Chacahua-La Pastoría en el sistema de información Biótica© Versión 4.2 y 4.5 respectivamente. El resultado de todo lo anterior es que se conoce lo siguiente de la ictiofauna oaxaqueña: la ictíofauna dulceacuícola está formada por 129 especies, 67 géneros, 34 familias y 16 órdenes; de estas especies, 117 son nativas y 14 son introducidas, de las segundas 3 son trasplantadas y 11 exóticas; las autóctonas están agrupadas en 57 géneros y 32 familias, cinco de las cuales son especies nuevas y tres de ellas no han sido descritas formalmente (Martínez y Gómez, 2006). En Parque Nacional "Lagunas de Chacahua" existen 65 especies, 47 géneros, 29 familias y 12 ordenes (Martínez-Ramírez, 2009). De la ictíofauna marina de la fauna de acompañamiento de la captura de camarón de alta mar en Golfo de Tehuantepec, se tienen 19 especies y 14 familias colectadas.

**The fish collection (ECOSUR), Chiapas, Mexico.**
**Rocío Rodiles-Hernández**

The Fish Collection (ECOSC) of El Colegio de la Frontera Sur (ECOSUR) in San Cristóbal de las Casas, Chiapas was founded in 1996 and has a current scientific wealth represented by 200 species, 5057 records and 27000 specimens of 350 localities fundamentally on the state of Chiapas and some records of Tabasco, Campeche, Oaxaca and Guerrero. 50 families (246 species) has been documented for Chiapas (Rodiles-Hernández et al in press), 33 are of marine and 17 freshwater origin. Nine families contain the 61% (149) of the total species richness (3 freshwater and 6 marine). The most diverse families are: Cichlidae with 16% (40 species, Poeciliidae with 11% (26 species), Scianidae with 7% (16 species), Carangidae and Ariidae with 6% (14 species) each one. At the present we have new projects in the Grijalva-Usumacinta basin (2008-2010) and other one for the Pacific Cost in Chiapas.

**The fish collection at ECOSUR-Chetumal**
**Juan Jacobo Schmitter Soto**

The ichthyological collection of ECOSUR at Chetumal was founded in 1986. Its geographical area of interest includes the nations and Mexican states in southern Mexico/northern Central America, with emphasis on the Yucatan Peninsula and the Mexican Caribbean. It has over 50,000 specimens, in more than 4000 lots, with 253 species (130 marine, 186 freshwater, 63 occurring in both environments), including two holotypes (Cryptoheros chetumalensis and Rocio gemmata) and 43 paratypes (the mentioned cichlids and also the pupfish Cyprinodon esconditus). There is an associated collection of live photographs, as well as ethanol-preserved tissues. Most of the freshwater ichthyofauna of the Yucatan Peninsula is well represented, as well as 50% of the marine fishes from the Mexican Caribbean.

**Integrated Ichthyologic data from Yucatan Peninsula**
**Maria Eugenia Vega**

Yucatan Peninsula exhibits unique conditions in relation with biodiversity because of its geographic position between the Gulf of Mexico and Caribbean Sea. Freshwater seeps and a wide platform with productive and heterogenic coastal habitats, promote the existence of great ichthyological species richness, including 437 species, from which 37 are Chondrichthys and 400 Actinopterygii. In 1984 we began evaluations about fish community structure in coastal and continental ecosystems such as Celestun and Rio Lagartos lagoons. We studied ecological interactions with mangrove ecosystem and the artisanal beach seine fishery, together with icthyological explorations along the Gulf of Mexico and Yucatan coast. We have contributed to the knowledge of fish biodiversity, and coastal ecosystem health, considering diversity and species richness and we found some taxa from Poecilidae, Cichlidae and Gerreidae families that require deep taxonomic evaluations through genetic and morphometric data. We recommend to carry on gathering and synthesis of taxonomical, ecological and biogeographic ichthyologic data and to make specific collections of those species that require taxonomical revisions (*Eucinostomus*, *Opsanus*, *Thorichtys* and *Micropogonias*)

**Freshwater fishes status in Mexico.**
**Salvador Contreras Balderas**

México is the southernmost country in North America, and extends into Central America South of the Isthmus of Tehuantepec. Its eastern limits are the Gulf of México and the Caribbean Sea, and in the Pacific Ocean along the West, including the Gulf of California in its entirety. The northern México is arid Temperate (Nearctic), while the rest is humid Tropical (Neotropical). Climate varies from extremely temperate deserts in the North, to tropical humid in the South. México has more than 500 described fish species, and some 30-40 still undescribed. There are around 2,500 marine species, 563 of them colonizers of the coastal flood plains. The burden of exotics has been growing also against freshwater forms. In 1904 only 4 species were recognized as exotics, increasing to 119 in 2008. The representative fish collections are at IPN, UNAM, and UANL. Time trend comparisons, show loss of native forms and increase of alien and/or colonizer species. The number of species reported by academics at some degree of risk have gone from 17 in 1963, to 198 in 2008. The trends in colonizers, exotics, and species at risk in Mexican fishes are parallel. One modern method of evaluation of the integrity and health of ecosystems is the Index of Biological Integrity (IBI) in geographical, or historical, applied to the Rio Grande/Río Bravo basin, USA and México, such as the Lower Rio Grande in the states of Tamaulipas/Texas and resulting in values between 0-70 (average 31) , both in the state of Coahuila, and 0-95 (average 42) in Rio Conchos state of Chihuahua. In summary the grades have been similar in all regions researched, from 70-80% in upper and middle reaches, to less than 35% in the lower reaches of West central basins down to under 15% near the Lower Rio Grande delta. The main problems plaguing the Mexican freshwater fishes are dewatering, pollution and alien species.

**DNA barcoding and north American freshwater fish diversity**
**Rick Mayden**

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 for identifying species for a variety of reasons. The barcoding fragment of COI is a short mitochondrial segment of 650 bp that serves as a conservative protein-coding gene and its utility ranges from being usable in degraded samples to situations where unique sequences exist for species where diagnostic morphological characters are absent and/or missing. 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, we have worked closely with those with the barcoding initiative in the generation of sequence data for several thousand specimens representing much of the diversity of North American freshwater fishes. We present our general findings as to both the usefulness of the gene for accurate species identification and for phylogeny reconstruction

**Advances in Mexican fish barcoding: freshwater and marine**
**Martha Valdez Moreno**

The fish fauna of Mexico includes some 2200 marine species and more than 500 freshwater taxa, while Guatemala has about 763 marine and 151 freshwater fish species, many endemic. In this study, we test the ability of DNA barcodes, based sequence diversity in the mitochondrial COI gene, to distinguish species from these two regions. In total, 242 species were analyzed including 179 species (1356 specimens) of marine fish (89 teleosts, 2 rays) and 63 freshwater fishes (546 specimens). Nearly 100% of the adult marine and freshwater material was identified correctly. Among freshwater fish, the genera *Astyanax*, and *Bramocharax* showed low divergences (<3%) between their species, but demonstrated a biogeographic consistency. Barcoding revealed previously overlooked taxa of the bonefish (*Albula*), *Floridichthys, Eucinostomus* and possibly of the freshwater *Ophisternon*. The only case of morphologically indistinguishable species was *Achirus lineatus* which split into two groups with up to 2.6% divergence. We conclude that barcodes were able to discriminate the marine and freshwater fishes from Mexico and Guatemala, reinforcing the discovery of overlooked taxa in several genera. Moreover, they validate the utility of DNA barcoding in the identification of larval fishes.

**The collection of fish larvae in ECOSUR and the barcoding**
**Lourdes Vásquez Yeomans**

La colección de larvas de peces de (ECO-CH LP) fue conformada en 1997 con material recolectado (1990 al 2002) en diversas áreas costeras de Quintana Roo, con redes estándar de plancton y fijado con formol. Actualmente la colección tiene 3900 registros curatoriales representados por 68 familias, 108 géneros y 125 especies. El pequeño tamaño de los ejemplares aunado a la falta de descripciones son factores limitantes en el avance taxonómico de larvas de peces. Como parte de un proyecto de colaboración entre NOAA y ECOSUR en enero de 2004 reiniciamos los muestreos de larvas de peces marinos utilizando artes de muestreo no convencionales (trampas de luz, redes de canal y colectores de columna de agua) en áreas costeras de Quintana Roo. Adicionalmente realizamos dos cruceros oceanográficos en aguas mexicanas, en marzo de 2006 (Cayo West Fl. hasta la frontera con Belice) y enero de 2007 (Cayo West Fl. hasta la frontera con Honduras). Las muestras fueron obtenidas utilizando redes MOCNESS y todo el material fue fijado en alcohol. Con la finalidad de establecer la identidad de larvas de peces costeras y oceánicas y posteriormente contribuir con descripciones larvales, en 2005 iniciamos un proyecto en FishBOLD utilizando la técnica de DNA-Barcodes. Hasta la fecha hemos secuenciado 730 larvas correspondientes a 137 especies; se presentan 3 ejemplos de resultados obtenidos: 1) complejo *Albula* spp., 2) complejo *Eucinostomus* spp. y 3) *Mycteroperca bonaci* (Serranidae).

**Barcoding Reef Fish Larvae**
**Benjamin Victor**

The utility of barcoding for studies of the early life history of reef fishes has only begun to be explored. Barcoding provides an excellent method to identify larvae to species and can also reveal the presence of cryptic species and illuminate phylogenetic relationships. I present a case study using the Caribbean gobies where a new species, *Coryphopterus kuna*, was first discovered as a larva and only later connected to an adult. In addition, the barcode analysis revealed the presence of cryptic species within the genus. Some of the cryptic species are new, such as the aptly-named *Coryphopterus bol*, some are old (the sequences confirmed a proposed resurrection of an invalid species), and some are regional variants from the edges of the range. In addition, barcoding also helps to identify the larvae of rare species, which are typically undescribed and usually remain undetected among conspecifics. I present an example of this with the identification of larvae of the rare and threatened giant cubera snapper from my collections in Caribbean Panama.

**Inventory of marine species and current status of collections of fish.**
**Alejandro Cotto**

It presents a summary table of the species identified in the Pacific and Caribbean of Nicaragua, state of exploitation of some species, profile investigations carried out fishing and the status of the two major collections of fishes (National University and Centroamericana University).

**Present of fish research and knowledge in Guatemala.**
**Rolando Wer**

As the Director of the project: Freshwater fish inventory in the main freshwater systems in Guatemala, started in the year 2000, I proposed a group of scientists which common interests in Guatemalan Fish studies. Working together with the BIOZENTRUM of the University of Würzburg (Germany, for advanced genetic studies) and the Tropical Aquaculture Laboratory of the UFL, through his Tropical American Ichthyologist supervisor, everyone in this team of friends has supported finacially his own participation in the study. Our difficulties, goals and limitations could be shared for future similar projects. Comparing our findings with anterior similar experiences, it shows that our endemic species are in danger of extinction. Water sources care and regulations should be also an issue in this kind of studies.

**Identifying Black Grouper (Mycteroperca bonaci) spawning aggregations of Belize**

**Mito Paz**

Black grouper (Mycteroperca bonaci) is a protogynous serranid that spawns in late winter (peak in February- March). In Belize, black grouper form many small

aggregations that move into deep water off of the reef face as spawning approaches. Spawning occurs in pairs that rush upward in the water column at dusk, spawn, and then rejoin the aggregation. During spawning, males exhibit a white-head phase, and spawn with dark-phase females. Blotched and light phases are also exhibited at all times of the year. Spawning occurs from the full moon to a few days after the last quarter moon, and occurs along a variety of reef formations, including elbows, romontories and linear shelf-edge reef. The formation of many small aggregations, rather than a few large ones, a slightly more protracted spawning period, and the feeding and diver avoidance behavior of black grouper has made it less vulnerable than some other groupers to fisheries in Belize, but it has been overfished in other parts of its range, and additional protection of spawning sites and habitats of uveniles is needed.

**Research of fishes in El Salvador**
**Ana Zetino**

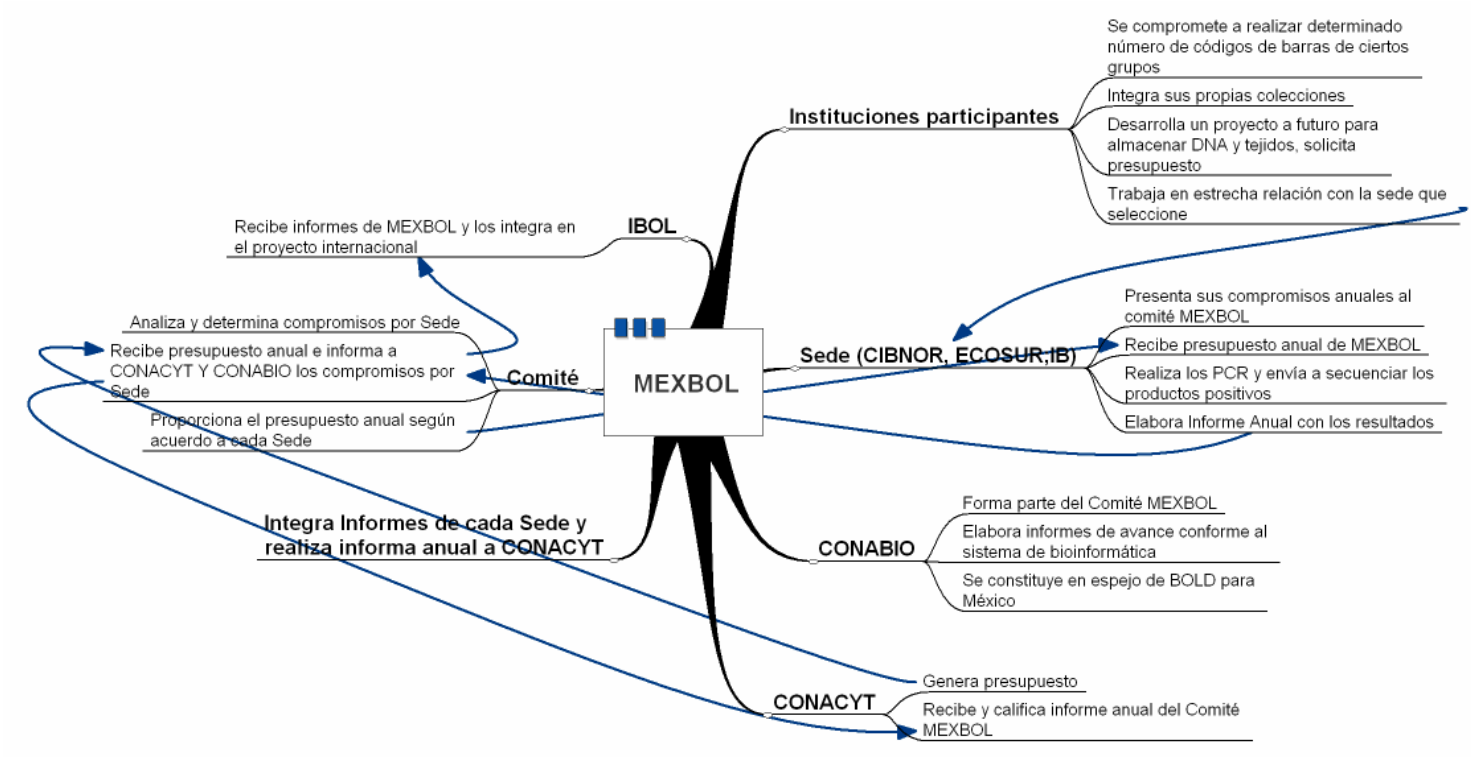
The goal of this talk is to present the investigations we are doing in El Salvador related to fishes, which are our concerns about these aquatic resources regarding their conservation and how to identify them by using new genetic techniques in order to contribute to the sustainable management of these organisms.

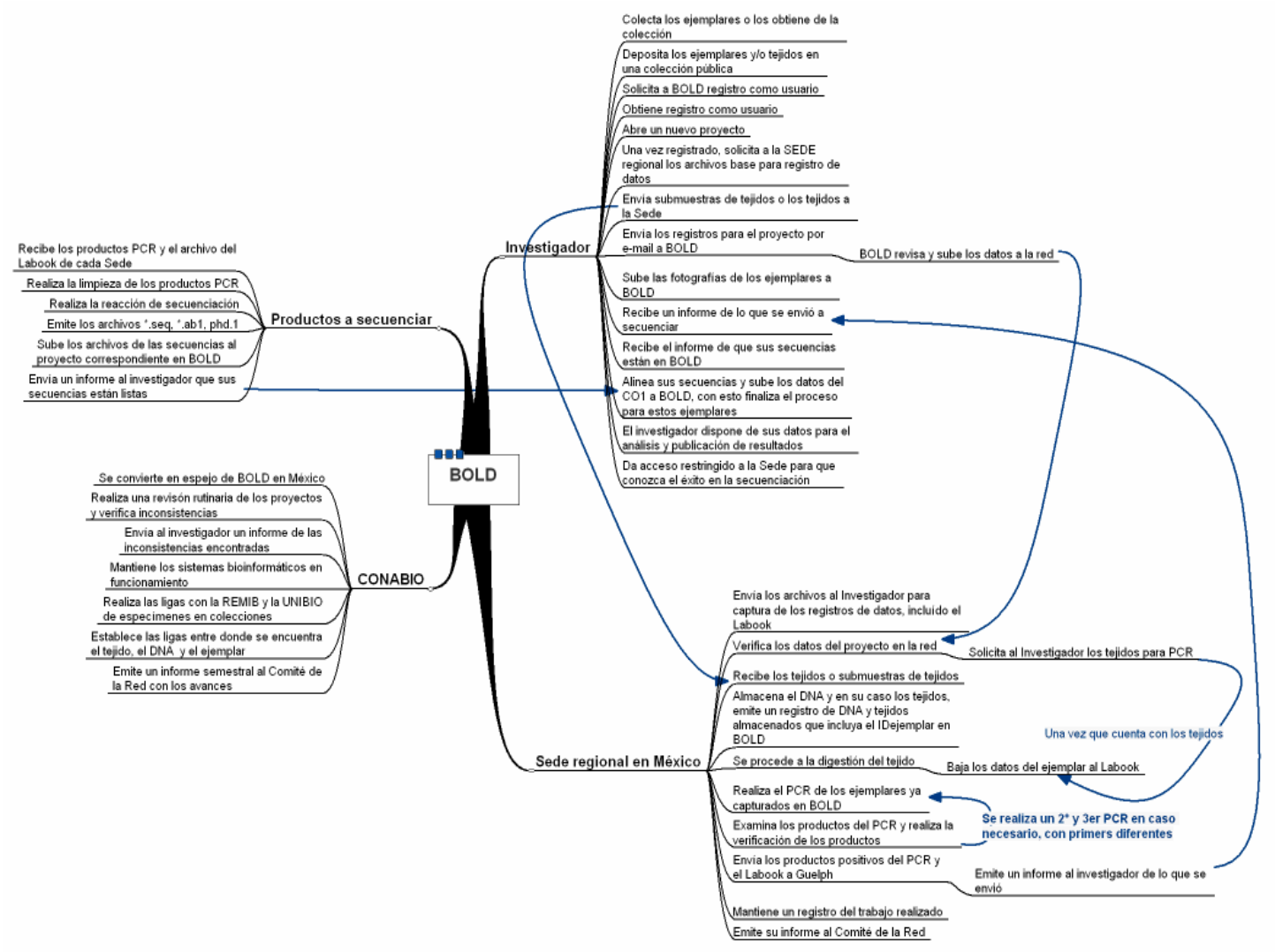
**FishBOL Canada – progress and current projects**
**Dirk Steinke**

This talk will present current results for Canadian FishBOL efforts. 700 Canadian species (4000 specimens) have been barcoded so far. The general overview will be followed by more detailed examinations of the different projects that are currently underway.

# FISH-BOL Participants

| Name | Affiliation | Contry | E-mail |
|---|---|---|---|
| Aguirre Villaseñor Hugo | Instituto Nacional de la Pesca | Mexico | Haguirre@ola.icmyl.unam.mx |
| Arena Ortíz Maria Leticia | UMIDI-SISAL UNAM | Mexico | mlao@hp.fciencias.unam.mx |
| Barba Macías Everardo | ECOSUR-Villahermosa | Mexico | ebarba@ecosur.mx |
| Campos Dávila Lucia | CIBNOR La Paz | Mexico | lcampos04@cibnor.mx. |
| Cázarez Carrillo Dalia | ECOSUR-Chetumal | Mexico | stella_cazarez@hotmail.com |
| Contreras Balderas Armando | Universiad Autónoma de Nuevo León | Mexico | arcontre@fcb.uanl.mx |
| Cohuo Colli José Angel | ECOSUR-Chetumal | Mexico | jacc85@hotmail.com |
| Dzul Panti Angel Manuel | Cinvestav IPN Unidad Mérida Yucatán | Mexico | adzul@mda.cinvestav.mx |
| Escobedo Cabrera Enrique | ECOSUR-Chetumal | Mexico | escobedo@ecosur.mx |
| Espinoza Sosa Engrih | ECOSUR-Chetumal | Mexico | |
| García Morales Alma | CONABIO | Mexico | almarotifer@yahoo.com.mx |
| García Rodríguez Francisco | CICIMAR | Mexico | fj_gr@yahoo.cpm |
| Guerrero Zárate Rocío | Cinvestav IPN Unidad Mérida Yucatán | Mexico | rguerrero@mda.cinvestav.mx |
| González Garza Idalia | Cinvestav IPN Unidad Mérida Yucatán | Mexico | bigzzg@gmail.com |
| González Paredes Wuiliam | EPOMEX | Mexico | wuilliam_gon@hotmail.com |
| Heredia Hernández Angeles | CINVESTAV-Mérida | Mexico | gelos_mda@hotmail.com |
| Hernández Armas Lidia | Universidad Autónoma Metropolitana -Xochimilco | Mexico | lha93@yahoo.com.mx |
| Hernández Ruiz Hortencia | UAM-Xochimilco | Mexico | hortencia0702@hotmail.com |
| Lizama Uc Gabriel | Universidad Nacional Autónoma de México | Mexico | glu@hp.fciencias.unam.mx |
| Landero Figueroa Marcela | Cinvestav IPN Unidad Mérida Yucatán | Mexico | mlandero@mda.cinvestav.mx |
| Martin Tun Mario | Cinvestav IPN Unidad Mérida Yucatán | Mexico | mmartin@mda.cinvestav.mx |
| Medina Quijano Humberto | Cinvestav IPN Unidad Mérida Yucatán | Mexico | hmedina@mda.cinvestav.mx |
| Nourisson Coralie | ECOSUR-Chetumal | Mexico | Coralie.nourisson@gmail.com |
| Ortíz León Héctor | Instituto Tecnológico de Chetumal | Mexico | oilh@yahoo.com.mx |
| Pimentel Camarillo Melissa | Instituto Tecnológico de Chetumal | Mexico | melissapimentelc@hotmail.com |
| Prado Cuellar Blanca | ECOSUR-Chetumal | Mexico | bprado@posgrado.ecosur.mx |
| Ramírez Eloy | Instituto Tecnológico de Chetumal | Mexico | eloy130811@hotmail.com |
| Reyes Avalos Aldo | Instituto Tecnológico de Chetumal | Mexico | tortu_spider@hotmail.com |
| Guadalupe Rincón | ECOSUR-Chetumal | Mexico | evenstar_890@hotmail.com |
| Rincón Sandoval Alberto | Cinvestav IPN Unidad Mérida Yucatán | Mexico | arincon@mda.cinvestav.mx |
| Rodríguez Gutiérrez Martha | UAM-Xochimilco | Mexico | rogm0211@correo.xoc.uam.mx |
| Sosa Vásquez Carolina | ECOSUR-Chetumal | Mexico | evenstar_890@hotmail.com |
| Terán Juárez Sergio Alejandro | Instituto Tecnológico de Chetumal | Mexico | bioente_tejuse@hotmail.com |


MEXBOL
Instituciones participantes
Se compromete a realizar determinado número de códigos de barras de ciertos grupos
Integra sus propias colecciones
Desarrolla un proyecto a futuro para almacenar DNA y tejidos, solicita presupuesto
Trabaja en estrecha relación con la sede que seleccione
IBOL
Recibe informes de MEXBOL y los integra en el proyecto internacional
Sede (CIBNOR, ECOSUR,IB)
Presenta sus compromisos anuales al comité MEXBOL
Recibe presupuesto anual de MEXBOL
Realiza los PCR y envía a secuenciar los productos positivos
Elabora Informe Anual con los resultados
Comité
Analiza y determina compromisos por Sede
Recibe presupuesto anual e informa a CONACYT Y CONABIO los compromisos por Sede
Proporciona el presupuesto anual según acuerdo a cada Sede
Integra Informes de cada Sede y realiza informa anual a CONACYT
CONABIO
Forma parte del Comité MEXBOL
Elabora informes de avance conforme al sistema de bioinformática
Se constituye en espejo de BOLD para México
CONACYT
Genera presupuesto
Recibe y califica informe anual del Comité MEXBOL


BOLD
Investigador
Colecta los ejemplares o los obtiene de la colección
Deposita los ejemplares y/o tejidos en una colección pública
Solicita a BOLD registro como usuario
Obtiene registro como usuario
Abre un nuevo proyecto
Una vez registrado, solicita a la SEDE regional los archivos base para registro de datos
Envía submuestras de tejidos o los tejidos a la Sede
Envía los registros para el proyecto por e-mail a BOLD
BOLD revisa y sube los datos a la red
Sube las fotografías de los ejemplares a BOLD
Recibe un informe de lo que se envió a secuenciar
Recibe el informe de que sus secuencias están en BOLD
Alinea sus secuencias y sube los datos del CO1 a BOLD, con esto finaliza el proceso para estos ejemplares
El investigador dispone de sus datos para el análisis y publicación de resultados
Da acceso restringido a la Sede para que conozca el éxito en la secuenciación
Productos a secuenciar
Recibe los productos PCR y el archivo del Labook de cada Sede
Realiza la limpieza de los productos PCR
Realiza la reacción de secuenciación
Emite los archivos *.seq, *.ab1, phd.1
Sube los archivos de las secuencias al proyecto correspondiente en BOLD
Envía un informe al investigador que sus secuencias están listas
CONABIO
Se convierte en espejo de BOLD en México
Realiza una revisón rutinaria de los proyectos y verifica inconsistencias
Envía al investigador un informe de las inconsistencias encontradas
Mantiene los sistemas bioinformáticos en funcionamiento
Realiza las ligas con la REMIB y la UNIBIO de especímenes en colecciones
Establece las ligas entre donde se encuentra el tejido, el DNA y el ejemplar
Emite un informe semestral al Comité de la Red con los avances
Sede regional en México
Envía los archivos al Investigador para captura de los registros de datos, incluido el Labook
Verifica los datos del proyecto en la red
Solicita al Investigador los tejidos para PCR
Recibe los tejidos o submuestras de tejidos
Almacena el DNA y en su caso los tejidos, emite un registro de DNA y tejidos almacenados que incluya el IDejemplar en BOLD
Una vez que cuenta con los tejidos
Se procede a la digestión del tejido
Baja los datos del ejemplar al Labook
Realiza el PCR de los ejemplares ya capturados en BOLD
Se realiza un 2° y 3er PCR en caso necesario, con primers diferentes
Examina los productos del PCR y realiza la verificación de los productos
Envía los productos positivos del PCR y el Labook a Guelph
Emite un informe al investigador de lo que se envió
Mantiene un registro del trabajo realizado
Emite su informe al Comité de la Red

# North and Central American Linkages for the DNA Barcoding of Fish

## Junio 5-6, 2008
## El Colegio de la Frontera Sur
## Chetumal

Los códigos de barras del DNA son una nueva técnica basada en una secuencia estandarizada del DNA mitocondrial, que sirve para identificar a nivel de especie a los peces y todos los animales de nuestro planeta.

Objetivos del taller

- Clarificar el concepto y aplicaciones de los códigos de barras
- Invitar a investigadores ictiólogos y estudiantes a participar en la campaña de Fish-Bol
- Uso potencial de los códigos de barras en áreas de potencial aplicación como pesquerías, acuacultura, prevención y control de especies exóticas y protección ambiental
- Problemas y controversias sobre el uso de los códigos de barras,
- Demostración de aplicaciones actuales de los códigos de barras en peces
- Búsqueda de oportunidades para obtención de recursos económicos para la investigación de los códigos de barras.

Costo del evento: ninguno

Cupo: Limitado

Idioma de las pláticas: Inglés

Los participantes sólo deberán registrarse y llenar un formato.

Interesados, comunicarse con Martha Valdez Moreno correo electrónico mvaldez@ecosur.mx o al teléfono (983) 83250440 ext. 4307

Se otorgará constancia de participación

Fecha límite de recepción de solicitudes de registro: **16 de mayo del 2008.**