



**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 diffusion 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](http://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](mailto:mvaldez@ecosur.mx)), Manuel Elías-Gutiérrez ([melias@ecosur.mx](mailto:melias@ecosur.mx)), Robert Hanner ([rhanner@uoguelph.ca](mailto:rhanner@uoguelph.ca)) and David Schindel ([schindeld@si.edu](mailto:schindeld@si.edu))

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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) Clarify 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. Biodiversity 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 % sequence 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](http://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 year. 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 possibilities 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 committed 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](http://www.conabio.gob.mx)) for specimens. The mode of participation will be through 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](mailto:melias@ecosur.mx)) ; CIBNOR, Sergio Ticul Alvarez Castañeda ([sticul@cibnor.mx](mailto:sticul@cibnor.mx)); IBUNAM, Patricia Escalante Pliego ([tilmatura@ibiologia.unam.mx](mailto: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](http://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](mailto: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](http://www.ibiologia.unam.mx)).

## Program

Thursday, June 5

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 Ichthyological 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 (Jacob Schmitter)
17:00-17:15	Coffee Break
17:15-19:00	Round table: Diversity and barcodes: Highlights and weakness. Chair: Bob Hanner

Friday, June 6

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	Ichthyology in Guatemala (Rolando Wer)
15:30-16:00	Identifying Black Grouper ( <i>Mycteroperca bonaci</i> ) spawning aggregations of Belize (Mito Paz)
16:00-16:30	FishBold Canada-Progress and current projects (Dirk Steinke, Videoconference)
16:30-17:00	Coffee Break
17:15-19:00	Round table: Linkages and collaboration possibilities for the fish barcoding from Central to North America Chair: Benjamin Victor

## FISH-BOL June 5-6

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