2nd FISH BARCODE OF LIFE WORLD CONFERENCE

Program & Abstracts

September 24-26, 2014

www.fishbolmx.org

Chetumal, Quintana Roo, Mexico

Contents

Wecoming remarks

Martha Elena Valdez Moreno
Chair of the Organizing Committee for 2nd Fish Barcode of Life World Conference

Chetumal, Mexico

On behalf of the Organizing Committee for the 2nd Fish Barcode of Life World Conference, I want to give my warmest welcome and sincere congratulations to all of you for your participation in this academic meeting.

Almost 10 years have been passed since the first publication on fish barcodes by our friend Bob Ward and collaborators, After this, a global campaign started and the Fish barcode of life (FISH-BOL) was created with the goal to coordinate a global effort of the barcoding fish. To speak about the importance of fish is useless; we all know the enormous importance of all fish in our lives, from the past to today. Just to mention something, here in the Caribbean they represent both a touristic attraction like the shark whale to a valuable resource as food, such as the grouper.

Now at this meeting, we have the opportunity to communicate with our colleagues from around the world, but in particular to the Mexican students. We all will have the valuable opportunity to know the results of our efforts, and the fundamental knowledge that the barcodes allow us to, not only to create the reference database, whose importance will play a key role in the future, but in learning when and where the fish spawn, where and what they feed on, the so-called functional ecology, and many other studies. Here is a firsthand opportunity to discuss the new challenges and applications that these beautiful animals represent to us. Also we have a golden opportunity to plan the future of our research by the end of 2015 when the International Barcode of Life Project will be complete.

This is also a good time to rediscover old friends and to create new collaborations for future research, and of course, to make new friends.

The aims are clear, the challenges immense, but we hope that your expectations for this conference will be accomplished, and enjoy your stay in Chetumal, a nice city, with lots of interesting places little known outside Mexico.

Congratulatory remarksss

Robert Ward, Robert Hanner and Claudio Oliveira

We welcome all presenters and attendees to this, the 2nd Fish Barcode of Life World conference. The first such conference was held two years ago, in Jeosu, South Korea. It proved to be a great success and we are certain this will be so for this meeting in Chetumal, Mexico.

Ten years ago, DNA barcoding using the mitochondrial COI gene was shown to be an effective new tool for fish identification. That initial study examined about 200 species of teleosts and elasmobranchs. Scientists around the world have now barcoded around 15,000 fish species, and hundreds of papers detailing their results have been published in international literature.

DNA barcoding is now globally recognized as an important tool for fish species identification, finding important applications in a growing number of areas as our understanding of their larval ecology and other aspects of their life histories, including food web structures. Scientists in at least 18 countries have applied this approach for seafood identification at the level of retailers and restaurants. Mislabeling has been found to be widespread and needs to be combated. Another major area that has been profoundly impacted by the adoption of DNA barcoding is that of taxonomy. Many new cryptic fish species have been recognized or flagged by DNA barcoding, and it is very clear that many more remain to be uncovered. Currently there are thought to be about 30,000 fish species, but it seems that this may well be a significant underestimate of the true number of extint species. There is clearly much more work that needs to be done to barcode all the world's fish species. We are perhaps about half way through this task but with dedication and perseverance the next ten years will see the goal reached.

The fish barcoding campaign is, of course, an international campaign. Meetings such as this one in Chetumal are very important for exchanging information and scientific advances, for maintaining links between existing collaborators and for setting up new friendships, new connections and new collaborations. Challenges still persist as the need to expand participation in Fish-BOL and to discuss structures for its future governance.

Finally, we warmly thank Martha Valdez-Moreno and her team for organizing and hosting this conference, and anticipate a most enjoyable and fruitful meeting.

Organizing Committees of the 2nd International Fish Barcode of Life Conference

Chair Martha Valdez-Moreno
El Colegio de la Frontera Sur, Chetumal, Mexico

International Organizing Committee

Claudio Oliveira (Instituto de Biociencias/UNESP , Brazil)
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Juan Díaz de Astarloa (Instituto de Investigaciones Marinas y Costeras, Argentina)
Juan J. Schmitter Soto (El Colegio de la Frontera Sur, Mexico)
Lourdes Vásquez-Yeomans (El Colegio de la Frontera Sur, Mexico)
Martha Valdez-Moreno (El Colegio de la Frontera Sur, Mexico)
Robert Hanner (Biodiversity Institute of Ontario, University of Guelph, Canada)
Robert Ward (CSIRO Marine and Atmospheric Research, Australia)

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Acknowledgements

To prepare a complex meeting like this, is not possible by one sole person, we need a team and support. Because of this reason, I want to thank our sponsors, in ECOSUR, our general director Mario González Espinosa, our branch director Hector Ortiz Arana, our Academic Director Juan Francisco Barrera, Department Director Laura Carrillo, Mexican Barcode of Life (MEXBOL) network, supported by Comision Nacional de Ciencia y Tecnología (CONACYT, grant 194025) specially to Manuel Elías Gutierrez, Comisión Nacional Para el Conocimiento y Uso de la Biodiversidad (CONABIO, grant MU010), and all private sponsors as Bordados Bernal, BMEDINA, MERIEQUIPOS, IMPERGRAFIC. Also, to all the people that assisted us, the Admninstrative staff of ECOSUR Chetumal, headed by Jose Chan Santamaria, all the Organizing Committe, and the staff, specially Jose Angel Cohuo Colli, Selene Morales Gutierrez, Yareli Cota Montaño, Ana Martínez Caballero, Enrique Escobedo and Arturo Domínguez García who prepared the web site and the book of abstracts.

Martha Elena Valdez Moreno
Chair of the Organizing Committee
2nd Fish Barcode of Life World Conference

Program

September 23th	
Registration (14:00 - 18:00) Fiesta Inn Hotel	

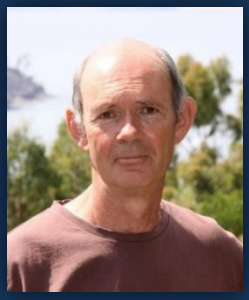
September 24th	
Registration	(07:30 - 08:50)
Opening Ceremony	(09:00 - 10:00)
Coffee Break	(10:00 - 10:30)
Keynote Speech 1	(10:30 - 11:30)
Chair person	**Juan de Astarloa**
10:30	**Robert Ward** Fish Barcoding: the First Ten Years
Session 1-2	(11:30 - 12:30)
11:30	**Manuel Elias-Gutierrez** An important application of DNA barcoding: protecting endangered species from international traffic
12:00	**Yuri Phedorovich Kartavtsev** Gene Introgression Throughout a Species Border: Concerns of Neo-Darwinism and DNA Barcoding
Lunch (12:30 - 14:00)	
Keynote Speech 2	(14:00 - 15:00)
Chair person	**Yuri Phedorovich Kartavsev**
14:00	**Dirk Steinke** DNA Barcoding and Education - a perfect match
Session 2-2	(15:00 – 16:00)
15:00	**Danilo Pimenta Neto Alves** DNA barcode reveals substitution of commercial seafood and fish products in Brazil
15:30	**Wazir Lakra** DNA barcoding of Indian sea food
16:00	**Laura Carrillo** Physical oceanography and barcode as tools of the early life history stages of fish in the Mesoamerican reef system
Poster mounting (16:00 - 17:00)	
Welcome Reception (18:00-20:00) Skybar Fiesta Inn Hotel	

September 25th	
Registration	(08:00 - 09:00)
Keynote Speech 3 Chair person	(9:00 - 10:00) **Lourdes Vásquez-Yeomans**
9:00	**Benjamin Victor** Status of Barcoding Coverage of Tropical W. Atlantic Shorefishes
Session 1-3	(10:00 - 10:30)
10:00	**Elva Maria Leyva-Cruz** Establishing the identity of fish eggs in the south of the Yucatan peninsula with barcodes
Coffee Break (10:30 - 11:00)	
Session 2-3	(11:00 – 12:00)
11:00	**Héctor Espinosa Salvador** Shark finning
11:30	**Zoila Raquel Siccha Ramirez** DNA barcode of tunas and allied species (Teleostei: Scombridae) in South America: effectiveness and limitations
Lunch (12:00 - 13:00)	
Keynote Speech 4 Chair person	(13:00 - 14:00) **Claudio Oliveira**
13:30	**Martin Juan Díaz de Astarloa** DNA Barcoding of Marine and freshwater fishes of Argentina: Progress after 8 years of research and future directions
Sesión 3-3	(14:00 - 14:30)
14:00	**Manuel Carranza Mendoza** Incipient Divergence in eyeless morphotypes of Dasyatis americana
Poster Session (14:30 - 17:00)	

September 26th	
Registration	(08:00 - 09:00)
Keynote Speech 5	(9:00 - 10:00)
Chair person	**Héctor Espinosa**
9:00	**Claudio Oliveira** Barcoding complex Neotropical fish groups
Session 1-3	(10:00 - 10:30)
10:00	**Ricardo Britzke** DNA Barcode of the genus Apistogramma (Teleostei, Cichlidae)
Coffee Break (10:30 - 11:00)	
Session 2-3	(11:00 – 12:00)
11:00	**Geremias Sánchez** Taxonomic and genetic analysis of species of the genus Micropogonias (Teleostei: Sciaenidae) in the Mexican Pacific
11:30	**Nir Stern** Evaluating species delineation in Sardinella and Spratelloides genera (Clupeidae) by aligning DNA barcoding markers parameters
Lunch (12:00 - 13:00)	
Sesión 3-3	(13:00 - 14:00)
Chair person	**Dirk Steinke**
Keynote Speech 6	(13:00 - 14:00)
13:00	**Robert Hanner** The Future of Fish-BOL
14:00	**Nicolas Bailly** Fish-BoL, DeepFin, FishBase and a new classification for Osteichthyes: a way forward for collaboration?
Farewell ceremony (14:00 - 14:30)	
Dinner at Riveros restaurant (19:00 -22:30)	

Keynote Speakers

Fish Barcoding: the First Ten Years



Speaker:
Robert Ward

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The FISH-BOL campaign was conceived ten years ago, in 2004, and in 2005 it was activated following a meeting of fish geneticists, taxonomists and interested parties at the University of Guelph, Canada. Now, according to BOLD's taxonomy browser, over 15,000 fish species have been DNA barcoded, from an average of about 11 individuals per species. This is nearly 50% of the world's known fish species. The great majority of fish species do indeed have species-diagnostic barcodes. Over the 10 year period, the efficacy and usefulness of the approach for identifying species has been demonstrated in many areas. These include consumer protection, health, trade, fisheries management and recreational fishing. Molecular ecology has benefited from the ability to identify eggs and larvae, and to better assess predator/prey relationships for food web descriptions. Barcoding has been immensely useful for taxonomy, providing a new and powerful tool for validating existing species, and flagging and then helping to describe new species. It also provides a standardized DNA sequence that can be incorporated into phylogenetic studies, thereby contributing to the science of systematics. While the FISH-BOL campaign has clearly achieved a great deal in its first 10 years, there still remains much work to be done: we remain a long way from attaining FISH-BOL's goal of barcoding all the world's fish species.

DNA Barcoding and Education - a Perfect Match

Speaker:
Dirk Steinke

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DNA barcoding provides an outstanding basis for science instruction because it bridges disciplines. As well, its workflows are simple enough that students can participate in all aspects of the analytical chain from specimen collection to data interpretation. The power of DNA barcoding in educational contexts was signaled in 2013 when this approach won the American Association for the Advancement of Science Prize for Inquiry-Based Science Instruction. The Biodiversity Institute of Ontario (BIO) is involved in the educational application of DNA barcoding through its School Malaise Trap Program and through the development of web-based instructional modules, mobile learning software and informatics tools. This presentation will showcase some of the projects that were developed and carried out at BIO over the last 3 years with an emphasis on transferable activities.

Status of Barcoding Coverage of Tropical W. Atlantic Shorefishes

Speaker:

Benjamin Victor[1]

Coauthors:

Lourdes Vásquez-Yeomans[2], Martha Valdez-Moreno[2]

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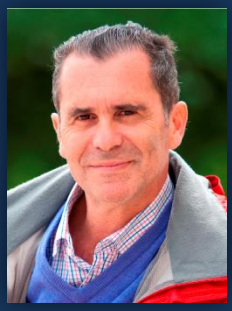
FISH-BOL has now sequenced about 80% of the approximately 1300 shorefish species in the Greater Caribbean region, and up to 90% of the coral-reef bony fishes). Almost all of the species have unique BINs, although a significant fraction either do not have a species identification or have an incorrect species ID. Three contributors account for the vast majority of species: ECOSUR, OSF/Victor, and the Smithsonian Laboratory. In our review of species coverage, species identifications were made primarily by confirmed voucher specimens, but many were identified by known nearest relatives, pairing with Pacific sibling species, and/or the process of elimination (quite feasible as we approach completion for many families). Those species still unbarcoded primarily consist of regional endemics (northern and southern edges in particular), soft-bottom and small cryptofauna, deep-water species, and species known from one or a few specimens (either rare or questionable taxonomy). Family coverage is lowest (about 50%) in uncommon cryptofauna, like Dactyloscopidae (17 spp.), Ogcocephalidae (14 spp.), and Ophidiidae/Bythitidae (47 spp.). Family coverage is highest among conspicuous and/or economically important families such as the Lutjanidae (18/18), Haemulidae (25/25), Carangidae (30/32), Pomacentridae (15/16), Labridae (20/21), and Serranidae (82/95). Other relatively large families with 100% regional coverage include Gerreidae, Apogonidae, Monacanthidae, Ostraciidae, and Diodontidae. Many families have a single BIN per species, often with widespread geographic coverage, but some have multiple lineages within species, typically, but not always, divided into geographic complexes; this pattern is most characteristic of the gobioids and blennioids (over 160 BINs so far in the Gobiidae with 109/134 spp.).

DNA Barcoding of Marine and Freshwater Fishes of Argentina: Progress after 8 Years of Research and Future Directions



Speaker:

Martin Juan Díaz de Astarloa

Coauthors:

Ezequiel Mabragaña, Mariano González-Castro, Juan José Rosso, Matías Delpiani

Affiliation:

Instituto de Investigaciones Marinas y Costeras (IIMyC), CONICET, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. Funes 3350, 7600 Mar del Plata, Argentina

Argentina has an area of ca. 2.8 million km^2 and the largest continental shelf in the world, with nearly 1million km². The country houses varied aquatic realms. The fish fauna comprises about 1000 species (freshwater and marine). In turn, about 150 species occur in the Argentine Antarctic sector. Since 2006 the active participation of Argentina in the Global Fishbol campaign began. Several research cruises have been carried out, building a large fish collection throughout the Argentine Sea, including coastal waters, the continental shelf and slope. Between 2011 and 2014 Antarctic cruises have been added and since 2010 we began sampling in inland waters, performing 13 field trips in 110 different sites. So far, the fish and tissue collection of the BIMOPE research group comprises more than 4000 specimens and about 490 species, representing ca. 45% of the fish species reported for Argentina. The first barcode sequences were obtained entirely in the Biodiversity Institute of Ontario, Canada. Since 2010 only sequencing was carried out there and the amplicons are developed in the iBOL reference laboratory in Mar del Plata. The barcodes generated were used to increase the BOLD reference library and to test the immensely valuable tool of barcoding for specific discrimination of Argentinean fish. As a result several publications including descriptions of new species and global studies of barcoding application to marine and freshwaters fishes have been produced. Future directions include mainly the onging collection of specimens to expand the reference library of Argentina´s fish fauna; research taxonomic studies in cartilaginous and bony fish using DNA barcoding as a complementary tool; to address socially relevant questions concerning market substitution and seafood traceability.

Barcoding Complex Neotropical Fish Groups

Speaker:
Claudio Oliveira

Coauthors:
Guilherme Jose Costa e Silva, Bruno Francelino Melo

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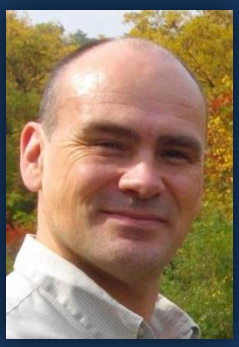
Some of the largest rivers in the world are found in South America and are inhabited by the richest freshwater fish fauna in the planet. Different from other parts of the world, the dominant orders in the Neotropical Region are Characiformes and Siluriformes, which represent about two thirds of all fishes inhabiting this region. Although many groups in these orders are composed of a few species, some very large families and genera occur. In the present study, we investigate two of the largest genera among characiforms (*Astyanax*, 142 species) and siluriforms (*Rineloricaria*, 63 species) with the main objective of testing whether the DNA barcoding technique is able to separate species in these large and complex groups. We analyzed 1690 COI sequences of *Astyanax* and found four large groups. These groups are quite distinct, with an average genetic distance of 13.5%. Inside the groups, the genetic distance ranged from 0 to 1.98%. The results show that 124 taxa comprise the genus *Astyanax*, using the 2% value as a threshold, a value very similar to that obtained with the ABGD method but different from that obtained with the GMYC method, which suggested the presence of many more groups. While many species could be well identified, several need deeper taxonomic and genetic studies to be confirmed or rejected. In *Rineloricaria* 228 sequences were analyzed. The genetic distance analysis revealed that 96% of the morphospecies (monophyletic cluster of all named specimens) differ from others by more than 2%; notwithstanding some morphospecies are genetically identical and a barcoding gap was not found. The GMYC analysis confirmed the identity of 43 of the 53 morphospecies, but suggested the presence of 70 putative species. Putative new species of both genera, as well as some putative synonyms were recognized, suggesting that the barcode technique can be very useful for species recognition in complex fish groups.

The Future of FISH-BOL



Speaker:
Robert Hanner

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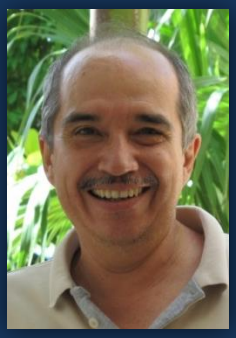
Over the last decade, the Fish Barcode of Life Campaign (FISH-BOL) has made great strides in building a molecular identification framework for the world's fishes. It has also revealed myriad new species and supports studies of trophic interactions, larval ecology, and other academic applications. FISH-BOL also supports a wide variety of applied projects involving environmental or "eDNA" detection of rare and/or invasive fishes and importantly, the identification of fillets and the detection of seafood fraud, which is implicated in Illegal Unregulated and Unreported (IUU) fishing practices. The socio-economic importance of FISH-BOL research requires that its proponents adhere to a higher level of data reporting than traditional academic research. Going forward, the FISH-BOL community will need to focus on several areas of endeavour, including: 1) increasing the depth and breadth of barcode coverage for fishes while focusing on type localities, 2) addressing conflict within the existing data through active annotation and curation routines, and 3) shift from typological to population and phylogenetic perspectives and barcode library construction. The latter will require an emphasis on increasing haplotype coverage for each species to facilitate reconstructing ancestral sequences for them. Preserving both reference sequences and identification query sequences will be needed to accomplish this task. Implications for the resulting knowledge base will be discussed as it pertains to combating IUU fishing, supporting accurate labelling, and environmental monitoring requirements.

Oral Presentations

An Important Application of DNA Barcoding: Protecting Endangered Species from International Traffic



Speaker:

Manuel Elías-Gutiérrez[1]

Coauthors:

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Illegal poaching and international trafficking of endangered species are some of the most common crimes around the world, representing tens of billions of dollars per year. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has listed those species endangered by this activity. Mexico is a megadiverse country, and the problem of ilegal traffic of endangered species is of special concern here. Some of these protected species are highly appreciated in the international markets as the shark fins, the totoaba fish bladder or the sea horses, just to mention a few. Smugglers appeal to all sorts of tricks to pass through the borders with these materials, and the DNA barcoding can be a great aid to identify the species from any kind of remain as hairs, scales, dried muscle, or live animals. With the final goal of helping control this trade, Google´s Global Impact Awards program financed an international project, the Barcode of Wildlife Project (BWP). The project currently involves four megadiverse countries (Mexico, South Africa, Kenya, Nigeria), and is coordinated by the Consortium for the Barcode of Life at the Smithsonian Institution in Washington, DC. In México, the project is coordinated by the Instituto de Biología of the Universidad Nacional Autónoma de México (IBUNAM), and involves the other academic institutions linked to barcoding (El Colegio de la Frontera Sur, Chetumal, ECOSUR, and Centro de Investigaciones Biológicas del Noreste, CIBNOR), CITES authorities (Comisión Nacional para el Concimiento y Uso de la Biodiversidad, CONABIO, Procuraduría Federal del Protección al Ambiente, PROFEPA and Secretaría del Medio Ambiente y Recursos Naturales, SEMARNAT), as well as the prosecution authorities (Federal Police and Procuraduría General de la República, PGR). The idea is to create a system to assist custom authorities to undoubtedly identify any species in trade and to provide a legal tool in any trial. For this purpose, in Mexico we have defined a list of 200 prioritary species, to build a reference library. This library will also include look-alike species and relatives of the prioritary species for a total of 1000 species and is being built following strictly standardized procedures for the sampling, storage of vouchers, and obtaining the DNA sequences. This library will be used by federal authorities as a reference to compare the evidence obtained of illegal trade events. Standard operating procedures are being defined for the sampling during authority's inspection events, transportation of samples under chain of custody and processing of the evidence in a wild life forensic laboratory.

Gene Introgression Throughout a Species Border: Concerns of Neo-Darwinism and DNA Barcoding

Speaker:

Yuri Ph. Kartavtsev

Affiliation:

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The author introduces the notion of hybrid and occurrence of hybridization estimates by means of molecular markers. Evidence on possible impact of gene introgression on species integrity, its evolutionary fate and consistency with the main modern paradigm, Neo-Darwinism, are considered.

Three main points will be discussed in the presentation. 1. What methods are most suitable for hybrid detection and estimation of genetic introgression and gene flow? 2. What facts on gene introgression are we talking about when different molecular markers are used, such as mtDNA and nDNA? 3. Is there a concordance of molecular diversity among different taxa levels and certain modes of speciation and predominated biological species concept (BSC)? It seems that claims on a crush of the modern BSC paradigm due to wide-scale gene introgression are too premature, especially keeping in mind the long history of many hybrid zones. Contrary to that, evidence summarized in the literature shows that molecular genetic data are concordant with the BSC and Neo-Darwinism in general. It is clear that introgression exists, although even in a wide zone of *Mytilus* spp., for example, it may be quite restricted or be asymmetric, so holding intact at least the "source" taxa. If we accept that the sexually reproducing species in marine and terrestrial realms are introgressed, then we should recognize that the orthodox biological species concept, in terms of complete gene flow absence among species, is inadequate in a sense that many zoological species are not biological species yet. However, sooner or later they become such, a conclusion supported by genetic distance increase with taxa rank and lowest diversity at intraspecies level. These outcomes have great impact to iBOL - FISH-BOL science policy and species identification in particular.

DNA Barcode Reveals Substitution of Commercial Seafood and Fish Products in Brazil

Speaker:

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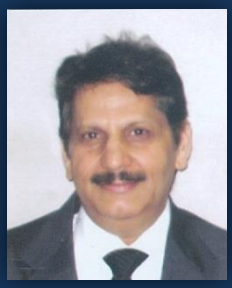
The occurrence of commercial substitutions in fish has been reported in several countries, including Brazil, mainly due to the absence of morphological characters in processed products. Genetic analyses are particularly important in cases of identification and certification as fillets, caviar, nuggets, or other processed products. The fraud by substitution for species of low commercial value by endangered species, or by species that could threaten human food security, are important to be identified by regulatory agencies. In order to verify the frequency of substitutions in processed fish products, 259 samples from supermarkets and restaurants in southeastern Brazil were analyzed. The extracted DNA was submitted to sequencing of approximately 650 bp of the COI gene, using the primers FISHF1 and FISHR1. The databases GenBank and BOLD were used as reference for molecular identifications. No fraud was found within the samples identified as tuna, shark, caviar, sardines, and tilapia. Twenty-six percent of all samples were considered substitution. The species with the highest substitution rate were hake (70%), cod (63%) and panga (43%). Samples of hamburgers, nuggets, kani, and whitefish (so named by restaurants of Japanese cuisine) could not be analyzed since there was not mention of fish species on their labels; however, five species were detected in these products demonstrating the variety of fish that are used as raw material in its production. Thus, we show that the technique of genetic barcode is a tool with real possibilities of implementation for the detection of substitutions in fish in nature and processed by the regulatory agencies in Brazil and that the BOLD database is the most reliable data, when compared to GenBank for these analyses. We thank the following funding agencies for financial support of the project: INCT, CNPq 573899/2008-8 and FAPEMIG APQ-0084/08.

DNA Barcoding of Indian Seafood



Speaker:

S.W. Lakra

Coauthors:

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Detection of mislabelling of raw and processed seafood is of global importance for reducing commercial fraud and enhancing food safety. Seafood identification and authentication has been carried out by different molecular methods including protein based and DNA based methods. Among DNA based methods, DNA barcoding has been successfully used for species identification and seafood authentication. In this study, DNA barcodes were generated for 32 species representing 13 families of Perciformes. The average genetic divergence values for within species, genus and family were 0.42, 13.91 and 18.05% respectively. Barcode gap analysis showed absence of overlapping between intra and interspecific divergence values. Intraspecific variation has increased several folds (15-20 times) after including conspecific sequences from different geographical locations. Presence of allopatric lineages and cryptic species was observed in several fishes with an Indo-Pacific region distribution. Further, we investigated the level of seafood mislabelling prevailing in India. Around 100 seafood samples including fresh, frozen, ready-to-cook, ready-to-eat and canned products were collected from different locations of India. The study reveals about 20% seafood mislabeling and it is much higher in restaurants (32%).

Establishing the Identity of Fish Eggs in the South of the Yucatan Peninsula with Barcodes

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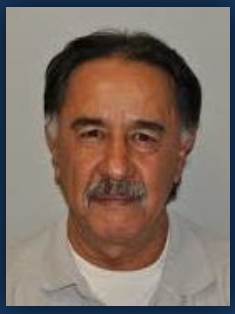
In the Mesoamerican Caribbean there are many spawning aggregation sites and nursery areas of fishes of different economic and ecological importance. It is almost impossible to identify fish eggs due to the lack of morphological characters. The gene cytochrome oxidase subunit I (known as "barcode") has been effective in connecting the early stages of fish development with the adults. This study aimed to recognize which species of fish spawn in waters of southern Quintana Roo (Mexico) and northern Belize using barcodes. The samples were collected during an oceanographic survey supported by ECOSUR, NOAA and the University of Miami. Seventeen stations located in the Mexican and Belizean Caribbean were reviewed. In total, 1390 eggs were collected and posteriorly identified as morphotypes; 300 of them were photographed and described. DNA was extracted and the COI gene was amplified and sequenced. We obtained 141 sequences that were compared with the Barcode of Life (boldsystems.org) database. We identified 32 species, in 34 genera and 23 families. Among the commercially important species were *Auxis thazard*, *Caranx hippos*, *Coryphaena equiselis*, *C. hippurus*, *Decapterus punctatus*, *Istiophorus platypterus*, *Kajikia albida*, *Katsuwonus pelamis*, *Thunnus atlanticus*, and *Xiphias gladius*. The station with the highest abundance of eggs presented 279 eggs. The most abundant species was *Nesiarchus nasutus*, with 446 individuals from six sampling stations. The species with the highest incidence were *Diplospinus multistriatus* and *Regalecus glesne*, present in eight stations.

Shark Finning



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One of the problems that arose from the meetings of the Barcode in Mexico project was the urgency of having a method which Mexican authorities could trust for detecting the "finning" (fin-cutting) of sharks. Shark finning includes tens of shark species, such as the whale shark, hammerhead, bigmouth, blue, and thresher sharks, and the fins usually are sold for food. Shark finning is more frequent in communities of low economic resources, because the sale of fins represents a much greater income than the average salary. Sharks are species of slow development and small populations and litters. Due to this, the finning (and consecutive death) of an adult individual has direct repercussions on the population health. In Mexico several species are in risk and at least four species are listed in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Therefore it is important to identify the species that are being exploited and finned. A solution that has been carried out along with the Profepa (Procuraduría Federal de Protección al Ambiente) was the sampling of confiscated tissues from which the barcode sequence was obtained. The barcoding of shark fin tissues were identified as three target species of the Mexican BARCODE list, genera *Sphyrna*, *Carcharhinus*, and *Mustelus*. These sequences will help in the conservation of sharks.

DNA Barcode of Tunas and Allied Species (Teleostei: Scombridae) in South America: Effectiveness and Limitations

Speaker:
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The tunas, mackerel, and bonitos belong to family Scombridae, which consists of 15 genera and 50 species of pelagic marine fishes of ecological importance, being at the top of the food chain, and high commercial interest, being caught in all the oceans of the world. For a long time the identification and separation of some of the species within the genus *Thunnus* have been almost impossible, due to morphological similarity and genetic introgression between some of the species. Previous studies suggest that the best molecular marker for the separation of species within *Thunnus* could be the D-loop. Considering this, we apply the technique of DNA barcode in combination with the D-loop to check if those markers could be used as a tool for the identification of the species belonging to the family Scombridae, particularly those often caught off the coast of South America. A total of 20 species belonging to 656 samples were collected from Brazil, Venezuela, Colombia, and Peru to analyze the barcode and 322 samples were analyzed with D-loop. A neighbor-joining dendrogram of K2P distances including bootstrap analysis with 1000 replications was performed using MEGA v.5.0 software to provide a graphic representation of the patterning of divergence among species. The barcode sequence clearly discriminates most species in Scombridae (66.16%) with exception of the genus *Thunnus*, where there is no effective separation. However, the D-loop adequately separates all species within the family Scombridae with high bootstrap support (>80%). Here we reaffirm that the control region is efficient in the separation of genus *Thunnus*, serving as a complement to the barcode.

Incipient Divergence in Eyeless Morphotypes of *Dasyatis americana*

Speaker:

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Habitat use has been associated to variable eye sizes and types; high concentration of suspended organic and inorganic matter in coastal environments can obscure visual stimuli, forcing organisms to rely on non-visual senses. Phylogenetic analysis of the mtDNA COI gene distinguished two haplotypes (0.3-0.5% genetic divergence) of the southern stingray *Dasyatis americana*; one haplotype belongs to three eyeless individuals and the other to four regular ones. Morphological description and comparison of six eyeless to regular individuals showed contrasting morphological traits besides the lack of eyes, such as body color; in eyeless stingrays the ventral edge is spotted grey-black and the dorsal color is darker compared to the regular morphotype, and the pelvic fin in eyeless specimens is rounded and oriented to the sides of the body, whereas in regular stingrays the pelvic fin is trapezoidal and oriented towards the anteroposterior axis. This is the first report on reproductively functional eyeless individuals of this species or close relatives elsewhere, which live sympatrically with regular individuals in the Gulf of Mexico.

DNA Barcode of the Genus *Apistogramma* (Teleostei, Cichlidae)

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The genus *Apistogramma* Regan, 1906 is one of the most specious genera of the family Cichlidae. This genus is distributed in the basins of the rivers Amazon, Orinoco, Paraguay, lower Paraná, lower Uruguay and in coastal rivers of the Guianas. Currently there are 84 valid species in the genus and many others are known and waiting to be described. These facts can result in problematic morphological identification since the limit between species and populations is not clear. Thus, molecular markers like the COI gene as proposed by Barcode are very helpful in delimitation and identification of species. In this context, the objective of the study was to analyze the genetic variability of gene COI in populations of species of *Apistogramma*, to test the limits between these species, and to help in their identication. We sequenced the mitochondrial gene cytochrome c oxidase subunit I of 510 *Apistogramma* samples from different South American drainages. We performed an analysis of genetic distance by the neighbor joining method and the Kimura 2-parameter model. From our analysis we identified 74 clusters within this genera, some of which are composed of several different species and others of different populations of the same species. Considering only the valid names, the difference observed between different species belonging to a same cluster varies from 3% to 22%; moreover, the difference between different populations of the same species varies from zero to 9%. Based on our results, we found that some species have wide distribution, little morphological difference and a large genetic divergence between populations, while others have a wide distribution and a low genetic divergence. In addition, there are species that are endemic to a particular region and have low genetic divergence. With our results, we note that some species of the genus *Apistogramma* could be constituted by a complex of morphologically similar species and there is evidence of some new species.

Taxonomic and Genetic Analysis of Species of the Genus *Micropogonias* (Teleostei: Sciaenidae) in the Mexican Pacific

Speaker:

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Family Sciaenidae consists of 24 genera in the Eastern Tropical Pacific; within these, genus *Micropogonias* is composed by three recognized species. These species exhibit meristic characters which mostly overlap, making their identification difficult. The aim of this study was to differentiate the genus *Micropogonias* distributed in the Mexican Pacific by morphological and genetic analysis. For this purpose, samples of *M. altipinnis* (n = 45), *M. ectenes* (n = 37), and *M. megalops* (n = 43) were collected, meristic characters were compared, and discriminant analysis with morphometric characteristics was performed. The shape of the otolith of the three species was analyzed by discriminant analysis comparing 15 variables. For genetic analysis, sequences of COI and 16S of mtDNA and 28S of nDNA were obtained, percentages of genetic divergence were calculated and neighbor-joining trees were constructed. The meristic analysis indicated differences in the number of rays of the second dorsal fin between *M. megalops* and *M. altipinnis*. However, this feature overlapped between *M. ectenes* and *M. megalops*, showing a latitudinal gradient between the three species in this character and the number of gill rakers and length of the longest spine of the first dorsal fin. Discriminant analysis showed three morphological entities ($p < 0.00$); using the most significant features, an equation of classification to differentiate among the three species was generated. The discriminant analysis showed differences in the shape of the otolith ($p < 0.00$). The mean genetic divergences of COI among species ranged between 0-0.6%; these differences are smaller than those reported at the intraspecific level (1%). The mean genetic divergences of 16S and 28S did not show significant differences among sequences of the three species. The neighbor-joining tree showed a clade with 78% boostrap support; within this clade, *M. altipinnis* and *M. megalops* were located at the extremes of the clade, but *M. ectenes* shared haplotypes with both species.

Evaluating Species Delineation in genera *Sardinella* and *Spratelloides* (Clupeidae) by Aligning DNA Barcoding Markers with Traditional Morphological Parameters

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Despite their high commercial importance and massive worldwide exploitation, our knowledge of the taxonomy and evolutionary relationships within the genera *Sardinella* and *Spratelloides* is still not fully elucidated. While integrating DNA barcode methodology and traditional morphology tools within these clupeids, we uncovered four states of taxonomic findings, two of which posed conflicting conclusions and two congruent outputs: (1) a description of a new cryptic species, *Sardinella* n. sp., from the Gulf of Thailand, was constructed based on 4.8% average genetic divergence in COI (5.2% in cytb) and three differentiating morphological characters between type specimens of *Sardinella* n. sp and their closest congeneric, *S. gibbosa*; (2) Three geographically distant species, *Sardinella aurita* (Mediterranean and Atlantic Ocean), *S. brasiliensis* (western Atlantic Ocean), and *S. lemuru* (eastern Indian Ocean and western Pacific) revealed minimal congeneric genetic diversity (ca. 1.8% in COI) as well as no differentiating morphological characters. This finding supports a century-old, yet unaccepted suggestion, which treated *S. aurita* as a single widespread valid species. Incongruent outcomes: (3) *Sardinella aurita* and *S. longiceps* (Indian Ocean) revealed negligible interspecific genetic divergence (ca. 1.6% in COI), while showing significant distinguishing morphological characters; and the opposite state (4) was documented within the round herrings *Spratelloides delicatulus* and *S. gracilis*, showing significant genetic intraspecific divergence in distant populations (16% and 10% in COI, respectively) with no differentiating morphological characters. Our study demonstrates the complicated relationship between molecular and traditional taxonomy approaches. In order to clarify such inconsistencies, future study should employ additional discerning tools, such as behavioral, reproduction compatibility and ecological parameters.

Fish-BoL, DeepFin, FishBase and a new classification for Osteichthyes: a way forward for collaboration?

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In 2013, Betancur-R. et al. published a new phylogeny and classification of the Osteichthyes (bony fishes) on the basis of 21 molecular markers. A third iteration was performed on 1596 bony fish species (4% of all 31,800 species) representing 394 families (79%) and 67 orders (100%) and is available on the web (www.deepfin.org/Classification_v3.htm). FishBase is ready to make the corresponding changes at class, order and family ranks that are well defined, leaving other intermediary ranks for specialist discussion. At the era of the web, it is difficult to change independently common knowledge: Scientists must avoid confusion for users. Global fish initiatives (CofF, FoW, FishBase, Fish-BoL, IUCN, DeepFin, etc.) should work in closer collaboration to be able to update their classification in a short time span. Moreover, there are obvious areas where Fish-BoL, DeepFin and FishBase can collaborate. For example, about 100 families are not yet represented in the DeepFin analysis (see a list on the web), the network of Fish-BoL could be used to get more sequences. On the other hand, DeepFin could systematically sequence the CO-I for identification purposes, even if could not fit in this analysis. And FishBase ensuring the links between websites. Such a classification must be the result of an international collaborative work to keep the interoperability between global (CofF, FishBase, ASFIS, CoL, Fish-BoL, IUCN, CITES, WoRMS, FADA, EoL, …), regional (ITIS, ERMS, FaEU, STEP, …), and national (ALA, CaRMS, INPN, …) initiatives for the convenience of users.

Physical oceanography and barcode as tools of the early life history stages of fish in the mesoamerican reef system

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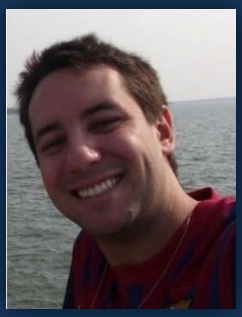
The Mesoamerican reef system (MRS), in the western Caribbean, represents the second largest coral reef barrier in the world. There have been several collaborative efforts in understand the spatial distribution of the early life history (ELH) stages of fishes. There is a clear agreement that the physical oceanographic processes in different scales are one of the key factors to understand the distribution and ultimate fate of the ELH stages of fish. This work is an attempt to provide a broad review of the knowledge of physical oceanographic processes linked to the ELH stages of fishes distribution and BARCODE, illustrated by observational physical oceanographic information from three oceanographic campaigns in the MRS and coastal data collection. Data collected during the campaigns included hydrographic (CTD casts), currents from shipboard ADCP and satellite tracked ARGOS drifters, while the coastal current observations were analyzed from Acoustic Doppler profilers. A regionalization of the MRS according to dynamics aspects such as circulation, water masses, mesoscale features and bathymetric aspects is suggested and also compared to preliminary results of the ELH of fishes distribution. The interaction of the oceanic currents with the coast such as the Yucatan Current played the more relevant important role in the northern region of the MRS, meanwhile the southern part of the MRS, weaker and variable currents determined a potential retention zone.

Poster Presentations

Preliminary Analysis of Fish Species of the Aquarium of the São Francisco River at the Zoobotanical Foundation of Belo Horizonte – FZB-BH using DNA Barcode



Speaker:

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Coauthors:

Thiago da Motta Carvalho[2], Denise Aparecida Andrade de Oliveira[1]

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The basin of the São Francisco River has great importance for the country not only by the volume of water transported in a semi-arid region, but also by its hydropotential and its historical and economic contribution to the region. At least 158 species of freshwater fishes are found in the São Francisco River. Among the 32 species presumably endangered in the State of Minas Gerais, 18 are there. The Aquarium of the São Francisco River Basin - FZB-BH is a partnership between the City of Belo Horizonte and the Brazilian Ministry of Environment. It has about 3000m^2 and it is the first to exclusively portray life in the São Francisco Basin. Besides being a place to visit, it is also aimed at carrying out studies and research. There are approximately 2800 fish from 79 species in 22 tanks and the "São Francisco Aquarium", with a capacity of 450,000 l. In order to identify and certify molecularly the fishes of the Aquarium, 50 specimens were collected, from the following species: killifish (*Hypsolebias radiseriatus*), falóceros (*Phalloceros uai*), serrudo (*Franciscodoras marmoratus*), pira (*Conorhynchos conirostris*), piaba (*Orthospinus franciscensis*), and acará topete (*Geophagus brasiliensis*). DNA was extracted and the sequencing of approximately 650 bp of the COI gene was done using the previously described primers FISHF1 and FISHR1. The sequences were deposited in BOLD and the analyses were conducted using the tools now available in its database. The average genetic divergences (K2P) among species, genera and families were 1.4%, 5.17%, and 10%, respectively. All specimens were grouped in separate clades, according to previous identification. The preliminary results indicate that it is possible to do genetic certification of the São Francisco Aquarium fishes using the DNA barcode technique. This study can also assist in the management and genetic conservation of species. We thank the funding agencies for support of the project: INCT, CNPq 573899/2008-8 and FAPEMIG APQ-0084/08.

Genetic Variation in *Skiffia lermae* (Cyprinodontiformes: Goodeidae) Using the Mitochondrial Gen Cytochrome Oxidase I (COI)

Speaker:

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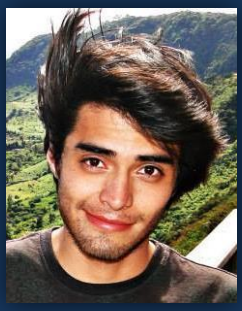
Skiffia lermae is distributed in the Lerma River basin, including lakes Cuitzeo, Pátzcuaro, and Zirahuén. The present study was conducted in order to know the genetic variation among these populations, and to infer the effects caused by the change on the hydrographic conformation of the basins in northern Michoacán during the Quaternary. Populations were sampled in La Minzita Spring in the Cuitzeo basin (CUI), Zacapu Lake in the Angulo River basin (ZAC) and Chapultepec Spring in the Pátzcuaro basin (PAT). In total, 27 sequences of COI of 627 pb were obtained, six pb were polymorphic and 621 invariant sites. The haplotype diversity (Hd) was moderately high, 0.5947, while the nucleotide diversity (π) was low, 0.0046. There were three haplotypes in total: Two haplotypes in CUI, one of them shared with PAT. The third haplotype was found only in ZAC. In addition, the average genetic distances ($\overline{(Dp)}$) were estimated, a $\overline{(Dp)}$=0.1% between CUI and PAT populations was found. The low differentiation and the shared haplotype between CUI and PAT populations, taking into account that the mutation rate estimated for Goodeids is 0.9% per million years, suggest that the populations were recently connected. Moreover, the genetic distance between CUI-ZAC and PAT-ZAC is $\overline{(Dp)}$=1.0%, suggesting that the population of ZAC has been isolated from CUI and PAT, for around a million years. The isolation of the populations was caused by the tectonic and volcanic activity in the region during the Quaternary.

Recognition of New Species of Fish in the Tropical Eastern Pacific



Speaker:

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There are millions of species on the planet, of which only a small percentage is known; therefore, it is necessary to generate knowledge of biodiversity. The use of DNA barcoding is a useful tool for species identification using DNA sequences, which allows to organize and record them into a genetic database. The Tropical Eastern Pacific (TEP) harbors a high diversity of fish species; this region is one of the most dynamic tropical marine environments, due to a complex system of ocean currents and habitat fragmentation of rocky reefs across its ca. 14,000 km length; therefore, this region appears as a model for the study of evolution of reef components. We present the identification of two possible new species out of a sampling across the TEP using mMitochondrial cytochrome oxidase I gene (COI). The results obtained by the analysis of molecular data (sequences and genetic distances) showed high levels of genetic divergence, 8.4% for *Scorpaenodes xyris* populations between individuals from the Gulf of California and central Mexico, whereas for *Anisotremus interruptus* the genetic distance was 3% between individuals from Galápagos Islands with respect to those from Mexico, Central and South America. Also, the Galápagos population is closer to *A. surinamensis* from the Atlantic than to the *A. interruptus* that inhabits across the TEP. Molecular data suggest the existence of species that have not been recognized until now. It is recommended to use other markers and morphological data in order to compare those results to COI.

Identifying Eucinostomus Larvae from Western Central Atlantic Using DNA Barcoding and Analysis of Morphological Characters

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The mojarras (family Gerreidae) are a common group of fish in tropical and subtropical coastal lagoons and they make an important contribution to commercial fisheries. In the Western Central Atlantic there are seven species of genus Eucinostomus, and while the morphological identification of the adult stage is relatively easy for most species, the identification of their larvae is still challenging. The larval stages of Eucinostomus are very similar among themselves in morphology and pigmentation, which has prevented their identification to species. In this context, we present results of DNA barcoding combined with morphological and pigmentation characters of Eucinostomus larvae with the objective of differentiating species. Larvae were collected from coastal samples (southern Florida, USA and throughout the Mexican Caribbean) and oceanographic cruises along the Mesoamerican Reef. A total of 150 specimens were sequenced using DNA barcode, corresponding to five species: E. argenteus, E. gula, E. jonesii, E. harengulus, and E. melanopterus. Morphometric features (standard length vs head depth and standard length vs head length) preliminarily reported differences between E. argenteus and E. harengulus, but diagnoses remains unclear for the remaining species. However, the pigment in the caudal region of the larvae may be a useful character; as in the case of E. melanopterus, whose dense pigmentation at the base of the caudal fin is unique; while E. argenteus usually shows four melanophores in the base of the caudal fin, whereas E. gula and E. harengulus have two and three, respectively. These characters are common in sizes between 7 and 14 mm. Through our investigation of genetically identified larvae, we suggest that patterns of tail pigment could be useful characters to facilitate the identification of larval stages of species of Eucinostomus when genetic analyses are not used.

Barcode of Life Suggests that *Canthigaster punctatissima*, *C. janthinoptera*, and *C. jactator* (Tetraodontidae) Are Synonyms

Speaker:

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Canthigaster is a circumtropical genus with 33 species; four of them are distributed in the Tropical Eastern Pacific (TEP). *Canthigaster punctatissima* is recognized as endemic but widely distributed in the TEP, *C. janthinoptera* is distributed in the Indo-Pacific and known as a vagrant in the Galápagos and Panama, and *C. jactator* is endemic to the Hawaiian Islands. The taxonomic characters for the identification of these species are not clear and most of them overlap, confusing the recognition of species in the TEP, even though color pattern around the eye is the most used character. The molecular marker cytochrome oxidase subunit I (COI) was use for the taxonomic determination of *Canthigaster* specimens along the TEP. Sequences of 36 individuals (593 bp) corresponding to samples collected from Mexico to Ecuador, including the Galápagos Islands, were obtained. Additionally, 11 GenBank sequences were used. The revision indicates the presence of all species in practically the sampled locations. Genetic distances obtained from molecular analyses are less than 0.4%, notwithstanding the geographic distance between the PET samples. When *C. jactator* (Hawaiian Islands) and *C. janthinoptera* (Africa, French Polynesia and Madagascar) obtained from GenBank are incorporated, the largest genetic distance is 0.28%, between *C. janthinoptera* and *C. punctatissima*. Comparing individuals collected to those registered in GenBank, similarity percentages ranged from 99-100% among *C. punctatissima*, *C. jactator*, and *C. janthinoptera*. We conclude that all the individuals genetically analyzed belong in one single species, therefore a taxonomic revision is needed in order to clarify its nomenclatural status.

COI Gene Confirms The Presence Of Bridled TriggerFish *Sufflamen fraenatum* (Balistidae) IN THE Coast of Mexico

Speaker:

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In the past, the identification of new locations for certain species based on a unique specimen could be uncertain. The use of molecular tools for identification of biodiversity, in conjunction with traditional taxonomy, allow more certainty in the identification of species. *Sufflamen fraenatum* has its original distribution in the Indian Ocean, East of Africa, and in the Pacific Ocean near Micronesia and Hawaii to the Pitcairn Islands, with a northern limit in Japan and to the South to Lord Howe Island. The only documented records for the Tropical Eastern Pacific date from 1899 (Revillagigedo Islands) and 1896-1900 (Mazatlán, La Paz, and Galápagos). However, taxonomic revisions in 1994 and 2008 discarded the record of this species in Mexico due to possible misidentification and uncertainty in distribution. In December 2010, a juvenile identified as *S. fraenatum* was collected on the coast of Michoacán, Mexico. Not much information to identify the juvenile stage is available, so in order to corroborate the identification, a fragment (650 bp) of the cytochrome oxidase subunit I (COI) gene was amplified and compared with all sequences of the species in GenBank (http://www.ncbi.nlm.nih.gov/genbank) and BOLD system (http://www.boldsystems.org). This comparison showed a 99.1% similarity to *S. fraenatum* specimens collected in West Opunoho, Mid Bay, Moorea Island, French Polynesia, South Pacific. Genetic analyses support the taxonomic identification of the specimen; therefore its presence is confirmed in the Mexican Pacific coast.

Cods for Sale

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The importation of marine products into Mexico has a long tradition dating from the discovery of America by Europeans. Norwegians traveled with dried cod and soon enough the cod market developed in southern Europe. Later, the first Spaniards to arrive in Mexico used this fish preservation method in their endeavors to America. The Atlantic cod *Gadus morhua* Linnaeus 1758 (bacalao or morue franche) is generally fished in northern countries, from the Gulf of Vizcaya in Spain-France, across the North Sea (or its connection with the Arctic), to Greenland and Canada in the American Atlantic. Cod have been an important wealth in the international market since 800 BC and the species *G. morhua* and *G. macrocephalus* are officially commercialized. In Mexico, cod is imported from Norway and the United States, and is traditionally eaten in Christmas and Easter Vigil. Due to the high price of the imported cod in Mexico, the sale of other species has been carried out. Among these species are sharks and other fish. In Mexico City there are several stores in which dry cod is available. The aim of this project is to corroborate the identity of the product sold as Atlantic cod by the obtention and DNA barcoding of dried-salted muscular tissue. We have seen that this product rarely is Atlantic cod, and in most of the cases the source are species near-threatened or that are incidentally fished.

Barcoding the Fish Fauna of the Uruguay River Basin, South America

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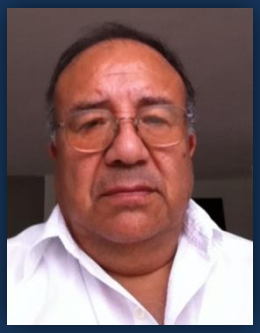
The Plata River Basin is the second largest drainage in South America after the Amazon . The La Plata River comprises four large sub-basins: Paraná, Paraguay, Uruguay and Plata Rivers. Ichthyofaunistic research is notoriously skewed since most efforts have been devoted to the Paraná River, the fish fauna of the Uruguay River being one of the poorest known so far. In this study we aimed to increase the knowledge about the richness of fish species in the Uruguay River by means of molecular analysis performed following the barcode protocol. We sampled 39 locations at middle and lower Uruguay River reaches, in Argentina. Our results revealed the occurrence of 92 different BINs grouped in seven orders, 26 families, and 52 genera. The COI sequence analyses and diagnostics revealed the existence of hidden diversity within some taxa previously supposed to be a unique taxonomic unit. Particularly, we detected two different BINs within emblematic species as *Rhamdia quelen* and *Salminus brasiliensis*, but also within the small *Phalloceros caudimaculatus*, the only species of this genus reported for Argentina. We also found strong evidence supporting the existence of two new species of the genus *Hoplias*. This was corroborated not only by a unique COI sequence among *Hoplias* specimens in the BOLD database, but also by means of meristic and morphometric analyses that unambiguously discriminated these putative new species from all other known species of Hoplias. Only one species within the genera *Rhamdia* and *Salminus* is reported for the Uruguay River basin in Argentina and both of them are subjected to be commercially exploited in the basin. Since we detected hidden diversity within these taxa, the possibility of overexploiting these resources can not be disregarded. Therefore, our results flag a very important task to be solved in order to properly manage and control the fishery effort exerted on these species.

Problems of Aquaculture in *Chirostoma* of the Mexican Altiplano Lands



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Ecological conditions that define most of the freshwater fish reservoirs in the Central Altiplano of Mexico depend on the quality of water that holds these ecosystems and that mainly comes from the contributions of the highly polluted Lerma River. *Chirostoma* (Swanson 1839) is a genus that according to the existing literature contains 18 species and five subspecies of fish known popularly as pescado blanco (white fish) and charales, that constitute a fragile resource of fishes of regional importance. Ongoing research points out that at present some taxa are already absent from their original locations, probably due to modifications such as reservoirs, the precarious quality of the aquatic environment where they survive, and overfishing. This work presents the actual situation of the fisheries and the ecology of *Chirostoma* in this region of Mexico, in order to engage the community in remediation practices and rescue of the still extant species in this genus. The aim of this presentation is to start barcode analysis for the molecular identification of these *Chirostoma* species.

Evaluation of ichthyoplanktonic community in the Neotropical reservoir through DNA barcoding

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Same Lima Costa

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Impacts caused by dams are responsible for drastic changes in the environment, causing loss of biodiversity, alien species introduction, and alterations in fish reproductive cycles. Assessing the reproductive success of fish populations is critical to develop conservation plans and one of the most effective ways of measuring such event is the identification and quantification of ichthyoplankton. However, the taxonomic identification may have some limitations, and the use of molecular tools help to a great extent to solve this. Thus, the aim of this work was to evaluate the composition of ichthyoplanktonic community in Rosana reservoir, Paranapanema River, upper Paraná River basin (Brazil), through DNA barcoding. Samples were collected from two main tributaries, Pirapó river and Pirapozinho river, and two lagoons related to the most productive period of the fish from November 2012 to March 2013. Sequences of COI gene (620 pb) from 72 samples were compared to sequences deposited in BOLD systems, which allowed the identification of 100% samples at specific level, resulting in 11 species, 11 genera, 7 families, and 3 orders. Two invasive species were identified, *Pterodoras granulosus* and *Plagioscion squamosissimus*, the latter with the highest frequency observed (50% of the identified species). The *P. squamosissimus* abundance in lentic environments can be attributed to high fertility rate in adult population, and larval predation of other species providing a competitive advantage. Larvae of species of migratory habit, such as *Pseudopimelodus mangurus*, a species in risk of extinction from which samples were taken only in Pirapó river, and *Pimelodus maculatus* were also identified, demonstrating the importance of maintaining lotic stretches for species conservation. The use of DNA barcoding was extremely informative for the identification of ichthyoplanktonic community thereby providing important information for ecological assessment and conservation of local species.

Chromatic Polymorphism in *Trichomycterus davisi* (Siluriformes: Trichomycteridae) Confirmed by DNA Barcoding

Speaker:

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Color patterns are characters highly conservative for *Trichomycterus* species, being commonly used for distinguishing and identification of its species. However, some studies indicate that there are considerable variations in these color patterns, such as variations between juveniles and adults, and related to microhabitats. In this context, the present study aimed to determine whether different color patterns observed in *Trichomycterus* collected in one stream representing different taxonomic units. For this purpose, 118 specimens were collected at Fazenda Monte Alegre Ecological Reserve, of which 88 were used for morphological analyses and 30 for DNA barcoding analyses. The results of the morphometric analysis by principal components showed no differences in the morphology of individuals analyzed, indicating only a trend towards association of a pigmentation pattern and the standard length of few individuals. The results of intraspecific K2P genetic distance (< 0.2 %) and neighbor-joining tree also did not show the existence of distinct groups within the species, demonstrating that the different patterns of pigmentation are polymorphic characteristics for T. davisi and are not sufficient to determine different species. However, the results support the hypothesis that the color variation can be related to different stages of development of organisms.

What Can DNA Barcoding Do for Fish Conservation in the Neotropical Region? An Empirical Example with Ichthyoplankton in the Upper Paraná River Basin (Brazil)

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The increasing use of hydroelectric power in large basins of the Neotropical region has caused severe impacts on fish, influencing reproductive success and endangering fish populations. Thus, identifying and protecting critical sites to fish reproduction is of paramount importance for conservation. Considering the ichthyoplankton identification complexity and DNA barcoding efficiency for neotropical ichthyofauna discrimination, the present study aims to evaluate the reproductive activity of fish species in Paranapanema River, upper Paraná River basin (Brazil), which is one of the watersheds most affected by dams in the neotropics. For this purpose, ichthyoplankton samples were collected in the middle part of Paranapanema River, in two tributaries of the Capivara's reservoir and lakes of Canoas I and Canoas II reservoirs, with conical nets (500 µm), during the months of highest fish reproductive activity (October to February), between 2012 and 2013. The genetic distance analysis of CO1 gene sequences (648 bp) allowed identification of 99.81% from 536 samples of eggs (293) and larvae (243) analyzed, belonging to 37 species, 27 genera, 15 families and four orders. The tributaries in the lotic portion of Capivara's reservoir showed the highest species richness (13 species), density of eggs (972.7 eggs/m³) as well as the higher frequency of migratory species. The identification of egg samples by DNA barcoding contributed about 70% of species richness; besides, seven species were exclusively found among egg samples. The results show the usefulness of DNA barcoding approach to identify species of freshwater ichthyoplankton, providing accurate and valuable information to management and conservation programs.

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