Introduction

Biodiversity loss is progressing rapidly; however, in many cases, its The causes are not entirely clear. The study of population genetics constitutes a fundamental tool for analyzing genetic variability between individuals and populations, as well as the distribution and evolution of alleles and genotypes, contributing key information for the conservation and sustainable management of species (Woodruff 2001; Terán-Martínez et al. 2021; Willi et al. 2022).

In fish, various studies have shown that genetic and genomic analyses They allow the identification of population risks and the assessment of their vulnerability to pressures. environmental. Previous research, such as that carried out on Atlantic salmon (Lehnert et al. 2019) and in common snook in the Usumacinta basin (Terán-Martínez et al. 2021), They demonstrate the value of these tools for biodiversity management and conservation of species of fishing importance.

In the state of Chiapas, the La Encrucijada Biosphere Reserve (REBIEN) encompasses the municipalities of Pijijiapan, Mapastepec, Acapetahua, Huixtla, Villa Comaltitlán, Huehuetán and Mazatán, and represents a region of high socio-environmental relevance due to its diversity biological and its economic importance for local communities (INECC 2023). Within this reserve, fishing activity focuses on white and blue shrimp, as well as in fish of high commercial value, among which the sea bass *(Centropomus)* stands out. sp.) (Gómez-González et al. 2012; Labastida-Che et al. 2013).

Previous studies have documented the presence of at least five species of the genus *Centropomus* in the REBIEN, of which *C. viridis, C. robalito* and *C. nigrescens* present greater socio-economic relevance and are known locally as "real" snook. “juelita” and “hocicón” (Gómez-González et al. 2012; Labastida-Che et al. 2013). No However, there is currently no genetic information available to characterize these populations within the reserve, which limits the understanding of its structure population and hinders the development of management and conservation strategies based on scientific evidence.

Therefore, the objective of this report is to describe the progress in the analysis of genetic variability, population structure, gene flow and connectivity intraspecific species of *C. viridis, C. ibalito* and *C. nigrescens* in the La Biosphere Reserve Crossroads, through the use of mitochondrial and nuclear DNA markers. These The results will help lay the foundation for future stages of the study and for decision-making. of decisions regarding conservation and fisheries management.

Activities

1. Sampling sites, collection and preservation of samples.

Samples of *C. viridis, C. robalito ,* and *C. nigrescens* were collected in the localities from El Carrizal, Topón, Salto de Agua, La Palma, Santa Isabel and Estero de Playa Grande (Figure 1), located on the southern coast of Chiapas, Mexico. All these localities are They are located within the REBIEN, where previous records have been documented of these species (Gómez-González et al. 2012).

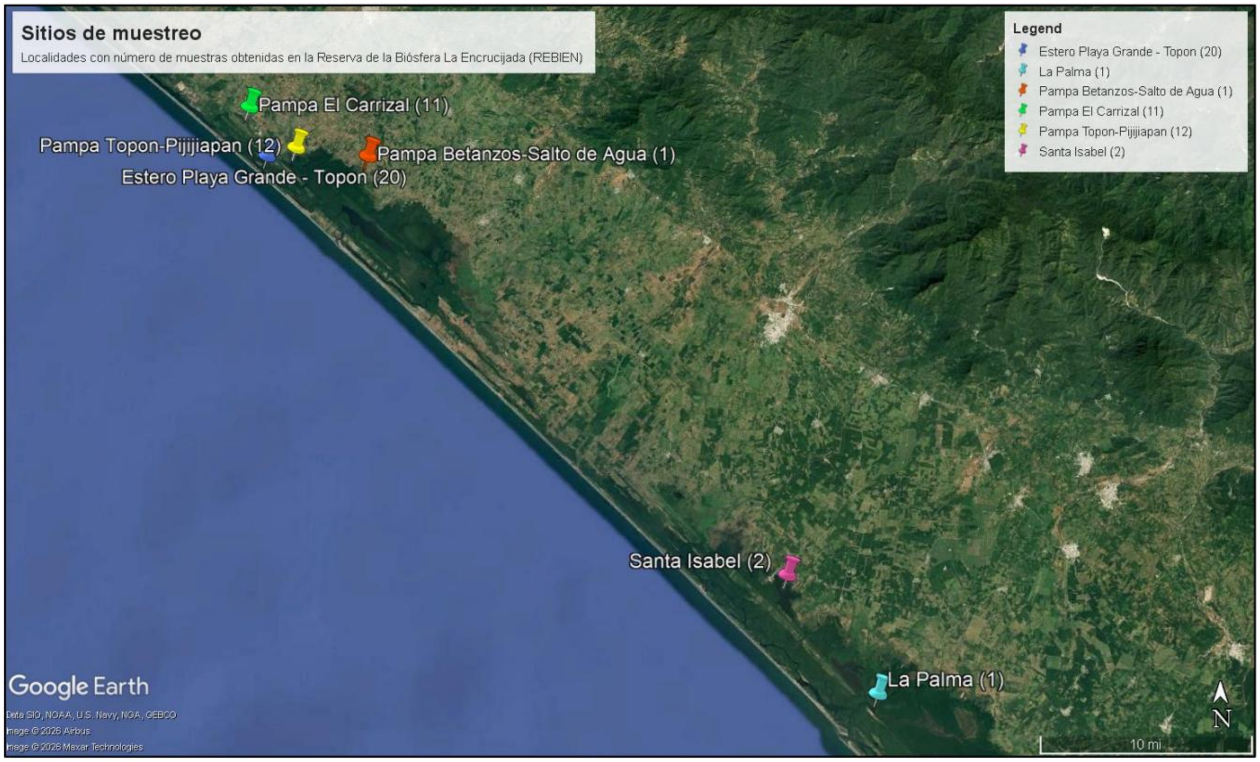

Figure 1. Sampling sites within the La Encrucijada Biosphere Reserve (REBIEN), showing the locations visited and the number of samples obtained in each one: Estero Playa Grande-Topón (20), Pampa Topón-Pijijiapan (12), Pampa El Carrizal (11), Santa Isabel (2), Pampa Betanzos-Salto de Agua (1) and La Palma (1).

The selection of sampling sites located in the REBIEN was based on collaboration of fishing cooperatives working with SmartFish AC (2025), a non-profit organization Key government entities are involved in this project. These cooperatives form part of a Fishery Improvement Project (FIP), so the results of this study will contribute directly to sustainable management the conservation of the region's fishery resources. Support was also provided by the technical staff of the National Commission of Natural Protected Areas (CONANP) in the snook harvesting activities and in liaising with fishing cooperatives.
premises.

Adult specimens were collected using fishing techniques such as cast netting, seine netting and harpoon with the support of local fishermen (Figure 2). A small fragment of fin of approximately 2 cm2 was taken from each individual (Nunes et al. 2015), in order to to preserve the commercial value of the captured fish, allowing their return to the fisherman or release, as appropriate. The collection took place during the period of July–December 2025.

Tissue samples were preserved in 95-100% ethanol (Nunes et al. 2015) stored in plastic vials, identified with unique keys that include the species and number of individuals (Figure 2). In addition, data were recorded supplementary data such as capture date, morphometric measurements (length, weight, body width, fin height, among others) and georeferenced photographs of the specimens (See Annex A).

Figure 2. Cast net capture technique, morphometric data collection and DNA sample collection from fin in *Centropomus.*

Subsequently, the vials were transferred and stored at -20 °C in the Laboratory of Environmental Biotechnology and Agroecology (LaBTAA) up to its processing.

2. DNA Extraction

The extraction of genetic material was performed using the Dneasy Blood & Tissue Cat kit. 69506 (QIAGEN, 2025). This kit is highly recognized for producing high-quality, free DNA. of contaminants and enzyme inhibitors. Therefore, the DNA obtained is suitable for PCR reactions and for genotyping techniques such as Southern blot blot, RAPD, AFLP and RFLP. The extracted DNA was stored at -20 °C until further use.

The quality and concentration of the extracted DNA was evaluated using a Nanodrop device. 2000 (Thermo Fisher Scientific 2009), which measured concentration and purity through absorbance indices (Lucena-Aguilar et al. 2016). DNA integrity was verified by electrophoresis in 1% agarose gels, run at 90 V for 30 minutes (Gödde et al. 2006).

3. PCR Amplification and Sequencing

The mitochondrial Cytochrome Oxidase Subunit I (COI) gene was amplified by PCR (NHGRI 2023), using the Fish F1 and Fish R2 primers (Ward et al. 2005; De Figueiredo-Filho et al. 2021), widely used in teleost fish due to their high Efficiency and specificity. This marker allowed the recovery of the maternal line of the individuals analyzed (Mahon 2007).

The PCR reactions were performed in a final volume of 25 µL, following standard amplification conditions. The thermal program was based on a protocol modified that incorporated a Touchdown PCR strategy to optimize the amplicon specificity (Mahon 2007; Li et al. 2011; De Figueiredo-Filho et al. 2021).

Amplification was verified by electrophoresis on 1% agarose gels, and the The products obtained were sent to Macrogen (South Korea) for purification and sequencing using the Sanger method, in both directions.

4. Bioinformatics and genetic analysis

The quality of the Sanger sequences was visualized and the initial regions were removed. and low-quality endings. Subsequently, the clean sequences were assembled and They were aligned using MEGA X v10.2.6 (Kumar et al. 2018). From these alignments, A preliminary genealogy was constructed in MEGA X v.10.2.6 (Kumar et al. 2018) by the Maximum Likelihood (ML) method applying the Kimura Model of two parameters(KP2) and the Adaptive Bootstrap (fast) was run to evaluate the support of the branches.

5. Workshops and dissemination of preliminary results

I participated in various workshops held on August 19 and 20, 2025 in fishing communities, as well as in outreach activities aimed at explaining the importance of the project and the scope of the research. Furthermore, participation was involved in the Presentation of a poster at the 1st Mexican Congress of Evolution: Science and Community in Evolutionary Biology.

Results

Sampling and identification

A total of 35 samples of *Centropomus* were obtained after the sampling carried out in the REBIEN localities (El Carrizal, Topón, Salto de Agua, La Palma, Santa Isabel and Playa Grande Estuary). Of this total, the molecular identity of 6 samples has been confirmed by BLAST analysis.

The detailed description of the samples and the status of their molecular identification is presented in Table 1.

Table 1. Summary of sampling and molecular verification of species of the genus *Centropomus.*

Sampling area	Species	Total samples	Confirmed (BLAST)	Unconfirmed
Biosphere Reserve - The Crossroads	*C. nigrescens C.*	12		11
	viridis C.	5	1	5
	ibalito	8	0	3
	Centropomus sp	10	5	10
	Total	**35**	0 **6**	**29**

Based on the collection of morphometric data from some captured individuals, the following is presented Table 2, which shows a summary of the recorded weights and sizes.

Table 2. Summary of morphometric data of the species of the genus *Centropomus* analyzed, including average total length, size range (minimum–maximum), the average weight and the capture methods used for each species.

Species	**Total Length Average (cm)**	**Size Range (Min - Max)**	**Weight Average (g)**	**Methods of Capture**
C. small snook	17.3	13.5 - 19.0	73.7	Cast net
C. viridis	41.6	39.0 - 45.5	436.0	Harpoon/Spotter, Mesh
C. nigrescens	44.0	32.5 - 57.5	420.3	Harpoon/Spotter, Mesh

DNA preservation and extraction

DNA was obtained from all 35 samples analyzed. DNA integrity was adequate. Defined bands were observed in 1% agarose gels. The concentrations of The extracted DNA was found to be within ranges suitable for molecular analysis. subsequent. Detailed information can be found in Annex D.

The detailed description of the samples and their DNA concentration is presented in the Table 3.

Table 3. Results of the quantification and purity assessment of the samples of DNA.

Clue	A260/280	Concentration (ng/ÿl)
NI-PA 01	1.82	494.9
RO-SI 01	1.81	458
RO-SI 02	1.81	458.5
RO-TO 01	2.05	158.3
RO-TO 02	2.04	149.6
RO-TO 03	1.81	465.5
RO-TO 04	1.81	459.4
RO-TO 05	1.81	494.9
RO-TO 06	2.09	79.5
CE-TO 01	2.02	189.1
CE-TO 02	1.99	232.9
CE-TO 03	2.05	127.8
CE-TO 04	2.06	154
CE-TO 05	2.06	153
CE-TO 06	2.04	186.1
CE-PG 01	2.03	133.7
CE-PG 02	2.06	153.5
CE-PG 03	1.93	60.6
CE-PG 04		321.2
VI-TO 03	2	207.3
NI-TO 03		71
NI-TO 04	2.01	104.5
VI-TO 04	2	176.9
NI-TO 05	2.08	169.8
NI-TO 06	1.97	169.1
NI-TO 07	1.99	154.6
VI-TO 05	2.25	258.4
NI-TO 08	1.99	181.4
NI-TO 09	1.97	219.3
NI-TO 10	1.99	182.5
VI-TO 06	2.01	99.5
NI-TO 11	2.04	194.8
NI-TO 12	1.96	202
VI-TO 07	2.04	108.3
NI-TO 13	1.99 2.02 2.06	135.6

PCR and Sequencing

Six PCR amplifications targeting the COI gene were performed. In the gels of Electrophoresis showed a band size of (~655-700 bp) in all individuals analyzed, which confirms the successful amplification of the fragment of interest. The The intensity of the bands was consistent between runs, which suggests a Efficient and reproducible amplification. Meanwhile, the negative control showed no bands. which indicated an absence of contamination.

Bioinformatics Analysis

During the second half of 2025, only 6 of the 35 were sequenced. samples, which were successfully identified through BLAST analysis of NCBI.

The genealogy presented (Figure 3) additionally included 17 sequences obtained during 2024 and the first half of 2025.

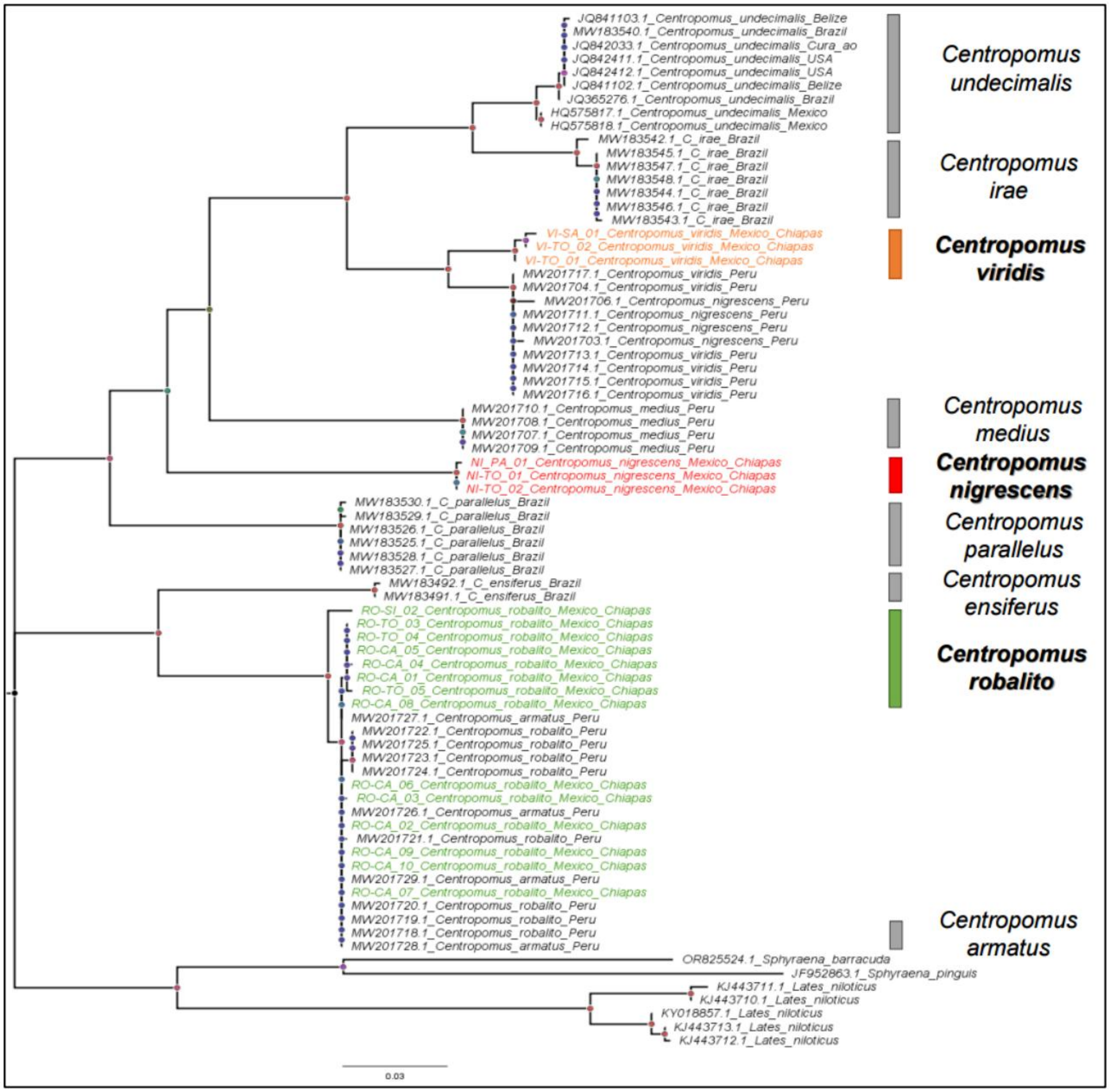

Figure 3. Genealogy based on sampled COI gene sequences of *Centropomus* in Chiapas (green, red and orange) and South America (SA), built using the method ML with *Latidae* and *Sphyraenidae* as an outgroup. The support values of each Nodes are represented by colors, where each color indicates a qualitative range of the Bootstrap value.

ML analysis showed that the *C. viridis* and *C. nigrescens* samples from Chiapas clustered differently from each other and with respect to the sequences South American (SA), indicating some differentiation between regions.

Conversely, the sequences of *C. viridis* and *C. nigrescens* obtained from GenBank (SA) They appeared intermingled, forming a single group. Meanwhile, C. robalito formed a well-defined clade that integrated specimens from Chiapas and South America, which This suggests a connection or low genetic differentiation between these regions.

Robustness was supported by the Adaptive Bootstrap (Fast) test. The values of The support of the branches was greater than 95%, therefore the relationships observed between The haplotypes are robust. This is clearly observed in the clade formed by *C. robalito.* In contrast, some of our samples of *C. virdis* and *C. nigrescens* They showed low branch support values.

The results of this preliminary phylogenetic analysis suggest the presence of groups distinct genetics of *C. viridis* and *C. nigrescens* in the Chiapas sampling area, to the once they confirm the Pan-American connection of *C. robalito* (Malcher et al. 2025; Ossaÿ Hernández et al. 2025).

Workshops and dissemination of preliminary results

On November 12, 2025, a proposal was presented to the fishing community of El Carrizal. A brief presentation explaining the purpose of the research and the methodology used for sampling, sampling locations and objectives of study (Figure 4).

Figure 4. Information session with fishermen from the community of El Carrizal for the Explanation of the project scope, capture methods, and sampling biological.

From November 18 to 21, 2025, we participated in the 1st Mexican Congress of Evolution: Science and Community in Evolutionary Biology, held at University City from the National Autonomous University of Mexico (UNAM), Mexico City, where he They presented the preliminary results obtained during the first stage of the research, in the form of a poster entitled: Population Genetics of Fish *Centropomus viridis, Centropomus ibalito* and *Centropomus nigrescens* in the Reserve of The La Encrucijada Biosphere (Figure 5; Annex B).

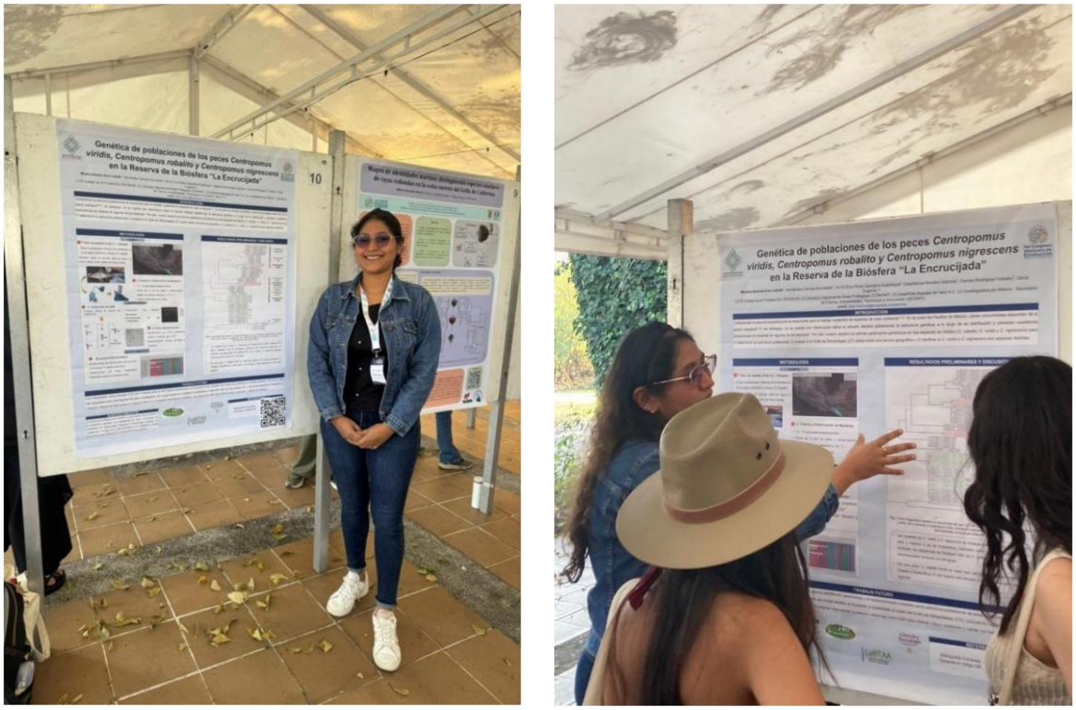

Figure 5. Presentation of the scientific poster entitled *Population Genetics of Fish Centropomus viridis, Centropomus ibalito and Centropomus nigrescens in the Reserve of The La Encrucijada Biosphere Reserve,* during the 1st Mexican Congress of Evolution: Science and Community in Evolutionary Biology.

Future work

Mitochondrial markers, such as COI, have limitations for delimitation taxonomic and the complete clarification of the population structure of these species. Therefore, in order to answer the questions raised in this research, it is It is essential to expand the sampling to locations west of the Gulf of Tehuantepec (GT), including Lagunas de Chacahua (Oaxaca) and Marismas Nacionales (Nayarit), included in Phase 2 of the study. It is also proposed to incorporate nuclear markers or next-generation genotyping techniques, such as GBS, in order to obtain higher genetic resolution and validate the findings obtained.

Additionally, it is necessary to strengthen sampling in the La Biosphere Reserve Crossroads (REBIEN) to complete the number of samples per species, as well as Complete the amplification processes using PCR and Sanger sequencing. This will allow the integration of the new sequences into the inferred genealogy and strengthen the interpretation of the results.

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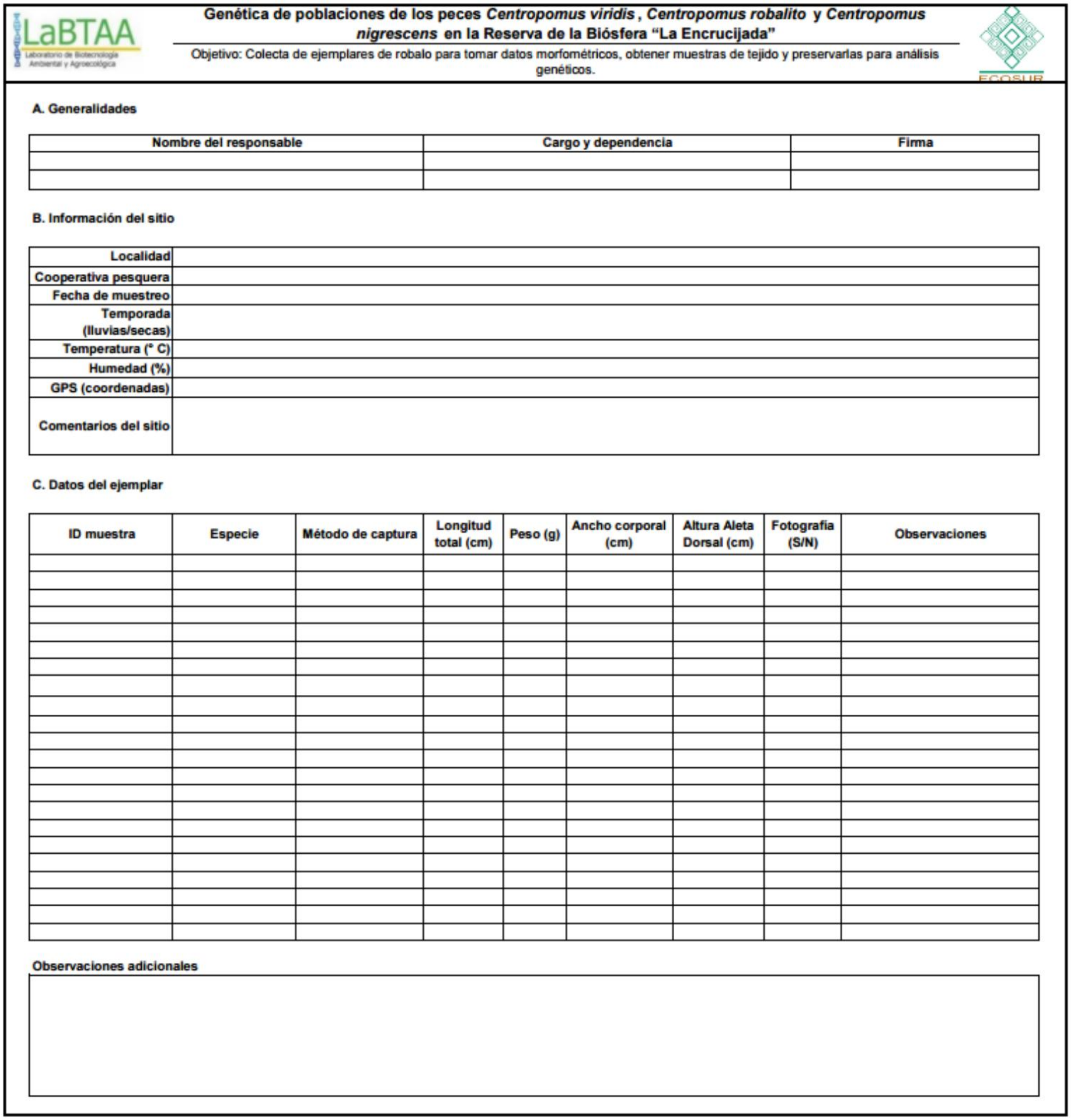
Annexes

A. Field format used for recording morphometric and environmental data of Centropomus sp.



LaBTAA
Laboratorio de Biotecnología Ambiental y Agroecológica

Genética de poblaciones de los peces *Centropomus viridis*, *Centropomus robalito* y *Centropomus nigrescens* en la Reserva de la Biósfera "La Encrucijada"

Objetivo: Colecta de ejemplares de robalo para tomar datos morfométricos, obtener muestras de tejido y preservarlas para análisis genéticos.

ECOSUR

A. Generalidades

Nombre del responsable	Cargo y dependencia	Firma

B. Información del sitio

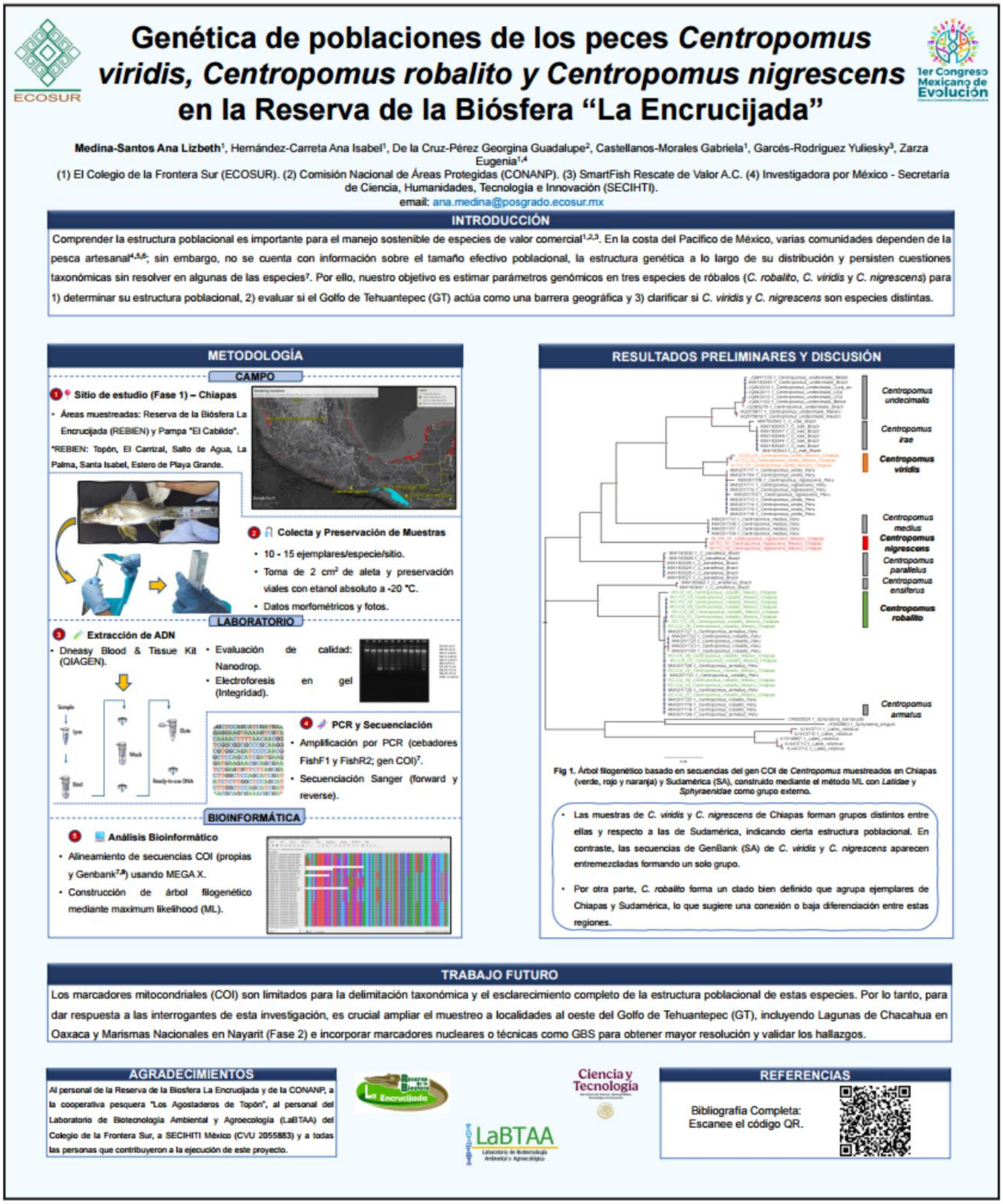
Localidad	
Cooperativa pesquera	
Fecha de muestreo	
Temporada (lluvias/secas)	
Temperatura (° C)	
Humedad (%)	
GPS (coordenadas)	
Comentarios del sitio	

C. Datos del ejemplar

ID muestra	Especie	Método de captura	Longitud total (cm)	Peso (g)	Ancho corporal (cm)	Altura Aleta Dorsal (cm)	Fotografía (S/N)	Observaciones

Observaciones adicionales

B. Scientific poster presented at the 1st Mexican Congress of Evolution: Science and Community in Evolutionary Biology.



Genética de poblaciones de los peces *Centropomus viridis, Centropomus robalito* y *Centropomus nigrescens* en la Reserva de la Biósfera "La Encrucijada"

Medina-Santos Ana Lizbeth[1], Hernández-Carreta Ana Isabel[1], De la Cruz-Pérez Georgina Guadalupe[2], Castellanos-Morales Gabriela[1], Garcés-Rodríguez Yuliesky[3], Zarza Eugenia[1,4]

(1) El Colegio de la Frontera Sur (ECOSUR). (2) Comisión Nacional de Áreas Protegidas (CONANP). (3) SmartFish Rescate de Valor A.C. (4) Investigadora por México - Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI).

email: ana.medina@posgrado.ecosur.mx

INTRODUCCIÓN

Comprender la estructura poblacional es importante para el manejo sostenible de especies de valor comercial[1,2,3]. En la costa del Pacífico de México, varias comunidades dependen de la pesca artesanal[4,5,6]; sin embargo, no se cuenta con información sobre el tamaño efectivo poblacional, la estructura genética a lo largo de su distribución y persisten cuestiones taxonómicas sin resolver en algunas de las especies[7]. Por ello, nuestro objetivo es estimar parámetros genómicos en tres especies de róbalos (*C. robalito*, *C. viridis* y *C. nigrescens*) para 1) determinar su estructura poblacional, 2) evaluar si el Golfo de Tehuantepec (GT) actúa como una barrera geográfica y 3) clarificar si *C. viridis* y *C. nigrescens* son especies distintas.

METODOLOGÍA

CAMPO

① Sitio de estudio (Fase 1) – Chiapas

- Áreas muestreadas: Reserva de la Biósfera La Encrucijada (REBIEN) y Pampa "El Cabildo".

*REBIEN: Topón, El Carrizal, Salto de Agua, La Palma, Santa Isabel, Estero de Playa Grande.

② Colecta y Preservación de Muestras

- 10 - 15 ejemplares/especie/sitio.
- Toma de 2 cm² de aleta y preservación viales con etanol absoluto a -20 °C.
- Datos morfométricos y fotos.

LABORATORIO

③ Extracción de ADN

- Dneasy Blood & Tissue Kit (QIAGEN).
- Evaluación de calidad: Nanodrop.
- Electroforesis en gel (Integridad).

④ PCR y Secuenciación

- Amplificación por PCR (cebadores FishF1 y FishR2; gen COI)[7].
- Secuenciación Sanger (forward y reverse).

BIOINFORMÁTICA

⑤ Análisis Bioinformático

- Alineamiento de secuencias COI (propias y Genbank[7,8]) usando MEGA X.
- Construcción de árbol filogenético mediante maximum likelihood (ML).

RESULTADOS PRELIMINARES Y DISCUSIÓN

Fig 1. Árbol filogenético basado en secuencias del gen COI de *Centropomus* muestreados en Chiapas (verde, rojo y naranja) y Sudamérica (SA), construido mediante el método ML con *Latidae* y *Sphyraenidae* como grupo externo.

- Las muestras de *C. viridis* y *C. nigrescens* de Chiapas forman grupos distintos entre ellas y respecto a las de Sudamérica, indicando cierta estructura poblacional. En contraste, las secuencias de GenBank (SA) de *C. viridis* y *C. nigrescens* aparecen entremezcladas formando un solo grupo.
- Por otra parte, *C. robalito* forma un clado bien definido que agrupa ejemplares de Chiapas y Sudamérica, lo que sugiere una conexión o baja diferenciación entre estas regiones.

TRABAJO FUTURO

Los marcadores mitocondriales (COI) son limitados para la delimitación taxonómica y el esclarecimiento completo de la estructura poblacional de estas especies. Por lo tanto, para dar respuesta a las interrogantes de esta investigación, es crucial ampliar el muestreo a localidades al oeste del Golfo de Tehuantepec (GT), incluyendo Lagunas de Chacahua en Oaxaca y Marismas Nacionales en Nayarit (Fase 2) e incorporar marcadores nucleares o técnicas como GBS para obtener mayor resolución y validar los hallazgos.

AGRADECIMIENTOS

Al personal de la Reserva de la Biosfera La Encrucijada y de la CONANP, a la cooperativa pesquera "Los Agostaderos de Topón", al personal del Laboratorio de Biotecnología Ambiental y Agroecología (LaBTAA) del Colegio de la Frontera Sur, a SECIHITI México (CVU 2055883) y a todas las personas que contribuyeron a la ejecución de este proyecto.

REFERENCIAS

Bibliografía Completa: Escanee el código QR.