

Morphological and DNA Barcoding Evidence Confirms the Presence of *Semaprochilodus taeniurus* (Prochilodontidae) in the Peruvian Amazon Basin

M. Ruiz-Tafur^{a, *}, J. Chuctaya^{a, b}, D. Castro-Ruiz^b,
C. Angulo^b, C. Garcia-Davila^b, and J. R. Garcia-Ayala^c

^a Fish Taxonomy Laboratory, Research Institute of the Peruvian Amazon, Iquitos, Perú

^b Laboratory of Molecular Biology and Genetics, Research Institute of the Peruvian Amazon Peruana, Iquitos, Perú

^c Ichthyology Laboratory, Zoology Department, São Paulo State University, São Paulo, Brazil

*e-mail: kruiz@iiap.gob.pe

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Abstract—*Semaprochilodus taeniurus* (Valenciennes 1821) are known to inhabit the middle and lower regions of the Amazon basin. This study presents the first record of this species in the Peruvian Amazon, in the Lizardo stream, a tributary of the Putumayo River, Loreto region. The specimens were identified using meristic, morphological, and morphometric features as well as DNA barcodes based on the mitochondrial *COI* gene. This new species record contributes to enhancing our understanding of fish diversity in the Peruvian Amazon.

Keywords: biogeography, diversity, Loreto, freshwater fishes, new record

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INTRODUCTION

The family Prochilodontidae, belonging to the order Characiformes, comprises 21 valid species (Castro and Vari, 2003, 2004; Melo et al., 2016; Fricke et al., 2023). All these species are known for their commercial and subsistence importance to the local populations in the areas where they inhabit (Goulding et al., 1988, 2018; Ribeiro and Petrere, 1990; Castro and Vari, 2004), with some of them being the most exploited and having the highest fishery landings in the main markets of the Amazon basin (Castro and Vari, 2003, 2004; Melo and Sidlauskas, 2017). The size of these species varies from 24 to 80 cm in standard length, and they are easily identifiable thanks to a disk-shaped mouth that can be turned outward, with multiple rows of small spoon-shaped teeth attached to fleshy lips (Castro and Vari, 2004). This group currently comprises three valid genera, which include *Ichthyoelephas* Posada-Arango 1909, *Prochilodus* Agassiz 1829, and *Semaprochilodus* Fowler 1941 (Melo and Sidlauskas, 2017). The genus *Semaprochilodus* distinguishes itself from the other genera within the family by the presence of dark stripes on the caudal and anal fins, less developed fleshy lips compared to *Ichthyoelephas* and *Prochilodus*, and the presence of a dark spot behind the opercular opening (Castro and Vari, 2004; Melo and Sidlauskas, 2017). Currently, the genus *Semaprochilodus* includes six valid species, among which *S. brama* (Valenciennes 1850) is

restricted to the Tocantins-Araguaia and Xingu river basins; *S. laticeps* (Steindachner 1879) is found in the Orinoco basin; *S. varii* Castro 1988 is distributed in the Maroni River basin in French Guiana and Suriname; *S. kneri* (Pellegrin 1909) and *S. insignis* (Jardine 1841) are distributed in the Amazon and Orinoco basins, with the latter having an unknown type locality; and *S. taeniurus*, which primarily inhabits the Amazon basin. Both *S. taeniurus* and *S. insignis* represent the most abundant species in the fishing records and are species that live in syntopy (Ribeiro and Petrere, 1990; Castro and Vari, 2003, 2004; Melo and Sidlauskas, 2017; Dagosta and De Pinna, 2019; Fricke et al., 2023).

Semaprochilodus insignis is found in lentic and lotic environments in Brazil, Bolivia, Colombia, Ecuador, Guyana, and Peru. On the other hand, *S. taeniurus*, commonly known as the “fine-scaled yaraqui”, was originally described from the upper river Negro basin by Valenciennes (1821). Its known distribution referred to the middle and lower portions of the Amazon basin, including Brazil and Colombia (Castro and Vari, 2003, 2004). It has been reported in the Brazilian Amazon in the Branco, Madeira, Negro, Tapajos, Trombetas rivers, and in the main channel of the Amazon River near the city of Manaus (Queiroz et al., 2013; Dagosta and De Pinna, 2019; Silvano et al., 2020). In the Colombian Amazon, this species has

only been recorded in the Apaporis River basin (Correa, 2003; DoNascimento et al., 2017).

In recent years, the DNA barcoding technique has become a very important method for species identification and verification of species records in aquatic environments due to its rapid and accurate production based on the cytochrome c oxidase subunit I (*COI*) gene. This project was initiated with the purpose of creating a simple diagnostic tool, grounded in a robust taxonomic knowledge compiled in the DNA Barcode Reference Library (Schindel and Miller, 2005). The progressive evolution of the DNA barcoding data system dates back to 2004 and was officially established in 2007 (Ratnasingham and Hebert, 2007), expanding its application to all eukaryotic species (Hebert et al., 2003; Miller, 2007). Since then, its use has increased worldwide, encompassing species description (Ota et al., 2020; Souza et al., 2023), species recognition at any life stage (i.e., both adults and immatures, including eggs) (Miranda-Chumacero et al., 2020; Mariac et al., 2022), new records (Guimarães et al., 2021; Chuctaya et al., 2021, 2023), and various applications related to the swift identification of specimens (Cutarelli et al., 2014; Martins et al., 2021). It has been highly valuable information included in recent studies in the Peruvian Amazon (Garcia-Davila et al., 2018, 2020). Hebert et al. (2003) proposed that intra-specific diversity of the *COI* gene in animals is significantly lower than inter-specific diversity and, therefore, it can serve as a standardized unique molecular marker for identifying unknown specimens. The need for this information has grown immensely due to the demand for projects focused on environmental DNA studies.

Despite the importance of *Semaprochilodus taeniurus* for the Brazilian fishery (Silvano et al., 2020), this species had not been recorded in the fishery of the Peruvian Amazon, as well as in recent reviews of the ichthyofauna for both the Loreto region (Meza-Vargas et al., 2021) and the Ucayali basin (Chuctaya et al., 2022), with its presence being unknown until before this record in Peru. Recent studies indicate that species identification has been achieved more efficiently by integrating morphological characteristics with gene sequence information (*COI*) (Chuctaya et al., 2021, 2023). The objective of this study was to investigate and record the Supper Amazon basin based on morphological, morphometric, and molecular analysis.

MATERIALS AND METHODS

Study Area and Sample Collection

Three specimens of *Semaprochilodus taeniurus* were captured using a 50-meter-long, 3-meter-high trap net with a 3" mesh opening, exposed in the water for a period of 12 h. The specimens were collected on 24 April 2022 in the Lizardo stream (02°15'27.1" S, 71°36'18.8" W) (Fig. 1), which is within the jurisdiction of the Bobona community in the middle Putu-

mayo River basin, Putumayo province, Loreto region. The specimens were anesthetized with eugenol diluted in 96% alcohol. One individual was selected from which 1 cm³ of muscle was extracted and preserved in 96% alcohol for molecular analysis. Subsequently, live photographs were taken of another specimen using a Nikon D3100 camera. Finally, the specimens were fixed in 10% formalin, complemented with injections of the same substance into the abdominal cavity, and kept for four days. Subsequently, the specimens were rinsed with plenty of water and preserved in 70% ethyl alcohol before being deposited in the Ichthyological collection of the Institute of Research of the Peruvian Amazon (CIIAP) for morphological and morphometric analysis.

Morphological Study

The specimens were identified using morphological, morphometric, and meristic analyses based on Castro and Vari (2004). Measurements were taken point-to-point using a digital caliper vernier (0.1 mm), and meristic character counts were conducted using a stereoscope. Measurements are presented as percentages of standard length (*SL*), and head measurements are represented as percentages of head length (*HL*).

DNA Extraction, Mitochondrial COI Amplification and Sequencing

DNA extraction was performed using the hexadecyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987), starting with 50 mg of muscle tissue. DNA amplification was carried out with the primers L5698-Asn by Melo et al. (2011) and FishR1 by Ward et al. (2005). The amplification was carried out in a total volume of 10 µL, containing 0.7 µL of Taq polymerase (1 U/µL), 1.0 µL of template DNA (100 ng/µL), 1.0 µL of Buffer 10×, 1.7 µL of dNTPs (2 mM), 1.0 µL of MgSO₄ (25 mM), 0.5 µL of each primer (10 µM), and 3.6 µL of ultrapure water. The temperature conditions were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 40 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The amplified products were separated by electrophoresis on 2% agarose gels. Sequencing was performed on a 3500XL Genetic Analyzer (Applied Biosystems) with the BigDye™ Tr v3.1 Cycle Sequencing kit following the manufacturer's instructions.

Data Analysis

The program BioEdit (Hall, 1999) was used to read and edit DNA chromatograms. The obtained *COI* sequences were verified using the Basic Local Alignment Search Tool (BLAST) search engine provided by the National Center for Biotechnology Information

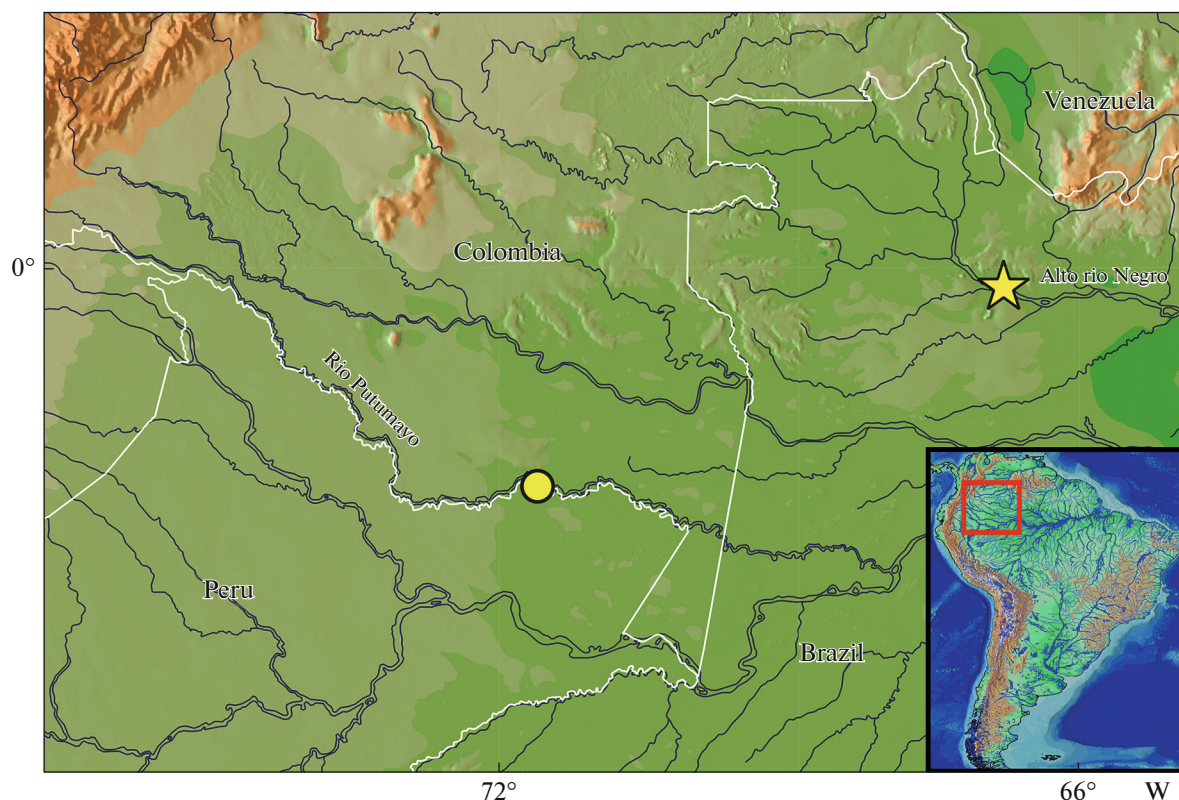


Fig. 1. Distribution of *Semaprochilodus taeniurus* (CIAP 2463) in the Amazon basin: (★) type locality, (●), new record in Putumayo River.

(NCBI) as one of the main public repositories for DNA barcode sequences. The Muscle procedure in the software MEGA11 (Tamura et al., 2021) was used for sequence alignment.

We estimated the best nucleotide substitution model with the MEGA 11 Software (Tamura et al., 2021). Models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), maximum likelihood value (lnL), and the number of parameters (including branch lengths) are also presented (Nei and Kumar, 2000). The best model selected was T92 + G (Tamura 3-parameter) + G (gamma distribution) (Supplement 1). The T92 + G model was used to calculate pairwise distances between specimens collected in the study area and several other specimens of the same species retrieved from NCBI, as presented in Table 1. The phylogenetic tree was constructed using the neighbor-joining (NJ) methods and the nucleotide substitution model T92 + G. The reliability of the branching tree was tested through bootstrap analysis using 1000 replicates in the MEGA11 software (Tamura et al., 2021). Additionally, a phylogenetic analysis using maximum likelihood (RAxML) was performed, this was run in RAxML-HPC2 on XSEDE 8.2.9 (Stamatakis, 2006;

Stamatakis et al., 2008) via CIPRES portal v3.3 (Miller et al., 2010). RAxML searches were conducted using 10 parallel runs, starting with a randomly generated tree. Branch support was assessed using the rapid bootstrap algorithm with 1000 replicates. A total of 31 *COI* sequences, representing 25 specimens from six species of the genus *Semaprochilodus* that constitute the ingroup, and 6 sequences belonging to the genera *Prochilodus* were used as an outgroup.

RESULTS

Semaprochilodus taeniurus (Valenciennes 1821)

Fig. 2

New record. CIAP-2463, 3 specimens, 189.1–194.2 mm *SL*, Lizardo stream (02°15'27.1" S, 71°36'18.8" W), Bobona community, middle Putumayo River basin, Putumayo province, Loreto region, Peru; 24 April 2022; collectors K.M. Ruiz-Tafur and S. Jean-Luca (Fig. 1).

Morphological analysis. The individuals collected from *Semaprochilodus* in the study area are characterized by having low intensity of dark pigmentation on the membranous opercular edge and on the exposed surface of the pectoral fin, features that distinguish them from *S. brama*, *S. laticeps*, and *S. varii*,

Table 1. Sequence information data extracted from GENBANK, including species name, tissue code, GenBank code, and references

Species	Tissue code	GENBANK	Reference
<i>Semaprochilodus varii</i>	GF15-041	MZ051468	Papa et al., 2021
	SU08-387	MZ050940	
	GFSU14-076	MZ051641	
	GF15-274	MZ051939	
	GFSU14-069	MZ051269	
<i>S. brama</i>	LBP 12776 41019	KX086769	Melo et al., 2016
	LBP 12807 41171	KX086770	
<i>S. laticeps</i>	LBP 12728	KF562436	Melo et al., 2014
	LBP 1383 12727	KX086748	Melo et al., 2016
	FMNH 113712	KX086778	
<i>S. taeniurus</i>	CIAP 2463 *	OQ472502	This study
	LBP 1691 12758	KX086751	Melo et al., 2016
	LBP 1691 12757	KX086750	
	LBP 1691 12759	KX086752	
<i>S. kneri</i>	LBP 3041 19140	KX086762	
	ANSP FISH 187277	KX086783	
<i>S. insignis</i>	1107	MH991706	Da Silva et al., 2019
	Jaraqui1	JN007735	Ardura et al., 2013
	J4**	FJ457765	Ardura et al., 2010
	1101	MH991717	Da Silva et al., 2019
	1108	MH991716	
	1109	MH991715	
	10694	MH991714	
	Jaraqui8	JN007742	Ardura et al., 2013
	Jaraqui10	JN007744	
	<i>Prochilodus nigricans</i>	D24	MN996700
D11		MN996699	
<i>P. lineatus</i>	ZQXKZ3	MT884695	Chen et al., 2021
	ZQXKZ2	MT884694	
<i>P. rubrotaeniatus</i>	SU08-358	MZ051928	Papa et al., 2021
	SU08-777	MZ051907	

(*) *Semaprochilodus taeniurus* species sequenced in this study, (**) *Semaprochilodus taeniurus* which should be corrected for *Semaprochilodus insignis*.

which have intensely pigmented black opercular membrane edges and exposed pectoral girdle surfaces. They also have 68 to 70 scales in the lateral line; 12 to 13 scales between the origin of the dorsal fin and the lateral line; 12 to 13 scales between the insertion of the pelvic fin and the lateral line, and 24(2) to 25(1) scales around the caudal peduncle, distinguishing them from the species *S. insignis* and *S. kneri*, which have a lateral line with 47 to 53 and 45 to 49 scales, respectively; scales between the insertion of the pelvic fin and the

lateral line with 9 to 11 and 7 to 9, respectively; and the number of horizontal rows of scales around the caudal peduncle is 18 to 22 and 16 to 20, respectively. These characteristics confirm the identification of this species as *S. taeniurus* and differentiate it from all known congeners (Fig. 2; Table 1).

Coloration in alcohol. The coloration characteristics of the specimens fixed in 10% formalin and preserved in 70% alcohol match the description provided by Castro and Vari (2004).



Fig. 2. *Semaprochilodus taeniurus* CIIAP 2463, 19.5 mm *SL*, Lizardo streamtributary of the Putumayo River: (a) live specimen, (b) preserved specimen. Scale: 10 mm.

Coloration in life. Overall bright silver coloration, darker in the dorsal portions of the head and body. Caudal fin is yellow with black stripes perpendicular to the direction of the dorsal and ventral lobes. Distal part of the dorsal fin and the anterior part of the anal fin are yellowish. Dorsal part of the iris is golden brown in color (Fig. 2).

Genetic analysis. The sequence alignment of the *COI* gene from 31 specimens produced 603 base pairs (b. p.) of nucleotides after removing ambiguous sequences near the primer ends, in which 467 positions were conserved, 163 were variable, and 161 were parsimony informative. Nucleotide frequencies were 29.4% thymine/uracil, 27.6% cytosine, 23.5% adenine, and 19.5% guanine. Additionally, 40 transitional pairs and 7 transversional pairs were determined, within the alignment, no stop codons were found. The *COI* sequence of *Semaprochilodus taeniurus* obtained in this study has been deposited in the open-access GENBANK repository with the accession code OQ472502.1.

The results obtained from BLAST sequences in NCBI matched the *Semaprochilodus taeniurus* with a 99.83% genetic similarity with *S. taeniurus* from to code KX086750.1, by Melo et al. (2016). Pairwise sequence distance of the *COI* sequence revealed an intra-specific variation of 0.18% between the specimens from the study area and the *Semaprochilodus taeniurus* specimens retrieved from GenBank (Table 1).

The NJ tree (Fig. 3a) showed that the five species, *Semaprochilodus insignis*, *S. vari*, *S. laticeps*, *S. kneri*, and *S. taeniurus*, formed separate groups. However, an exception was the specimen with the code FJ457765.1 previously identified as *S. taeniurus*, which ended up being grouped in the middle of *S. insignis* with a genetic distance ranging from 0 to 0.19%. This specimen presented a genetic distance of 11.32 to 11.72% with *S. taeniurus*, suggesting a possible identification error, and its correct identification should be *S. insignis* (Table 2). These results are supported by a phylogenetic analysis RAxML (Fig. 3b), where the *Semaprochilodus taeniurus* species cluster together. This analysis does not allow the differentiation between

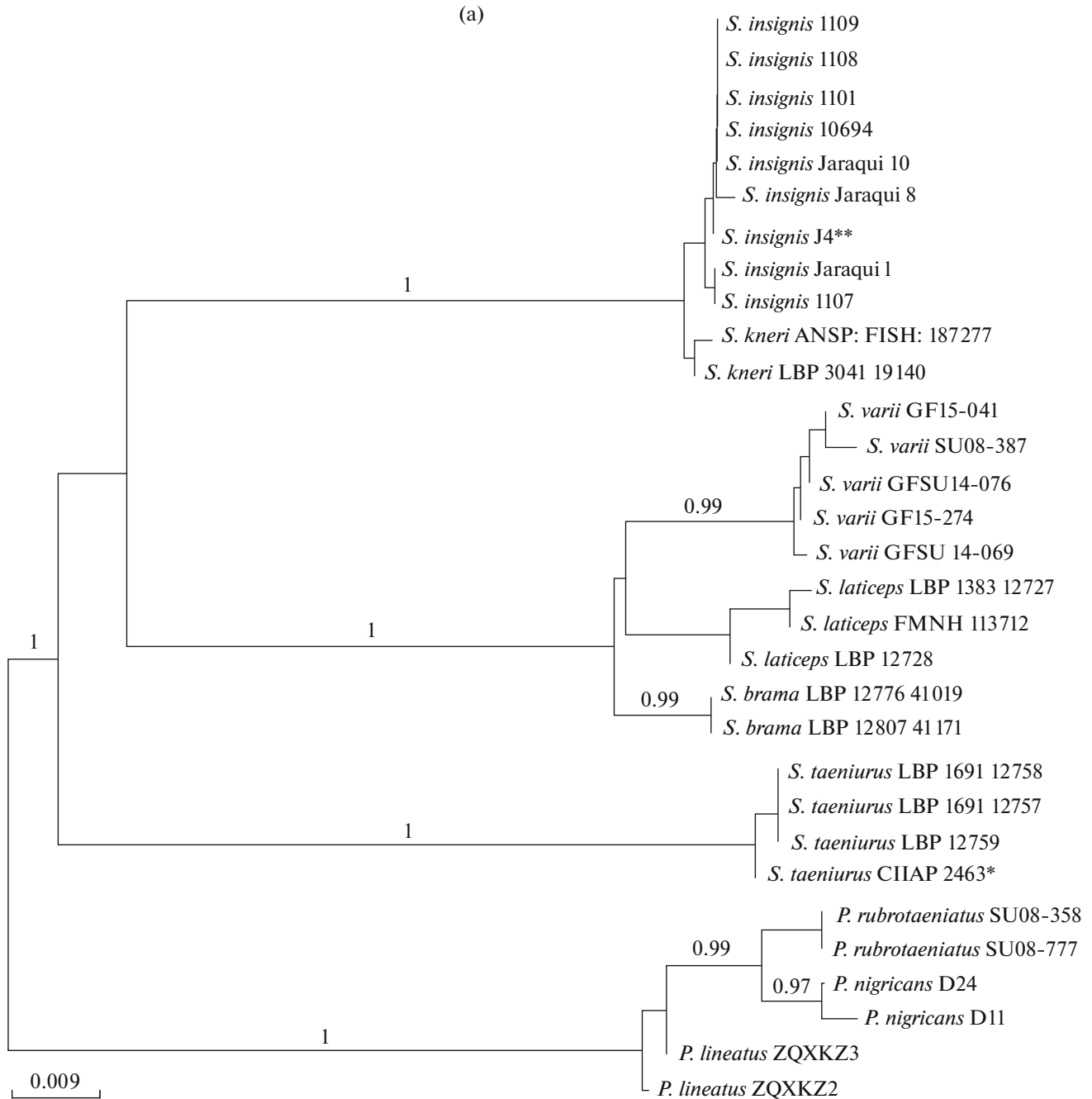


Fig. 3. *Semaprochilodus* Neighbor joining (NJ) phylogeny tree based on cytochrome c oxidase I (*COI*) gene sequences generated in MEGA 11 (a) and phylogenetic relationships of *Semaprochilodus* (and out-groups) supported by maximum likelihood (RAXML—GTRGAMMA) using *COI* sequences. The labels at the branch tips refer to the species name and accession number. The numbers on the branches refer to bootstrap values for each node. The *Prochilodus* genus were selected as outgroups. (*) *Semaprochilodus taeniurus* species sequenced in this study, (**) *Semaprochilodus taeniurus* which should be corrected for *Semaprochilodus insignis*.

S. insignis and *S. kneri*, necessitating the use of other molecular markers to distinguish these species.

Distribution. *Semaprochilodus taeniurus* was known to inhabit the middle and lower portions of the

Amazon River basin and its tributary rivers, including the Rio Negro, Rio Branco, Rio Madeira, and Rio Tapajos (Castro and Vari, 2004; Silvano et al., 2020). In the upper part of the Amazon basin, this species was

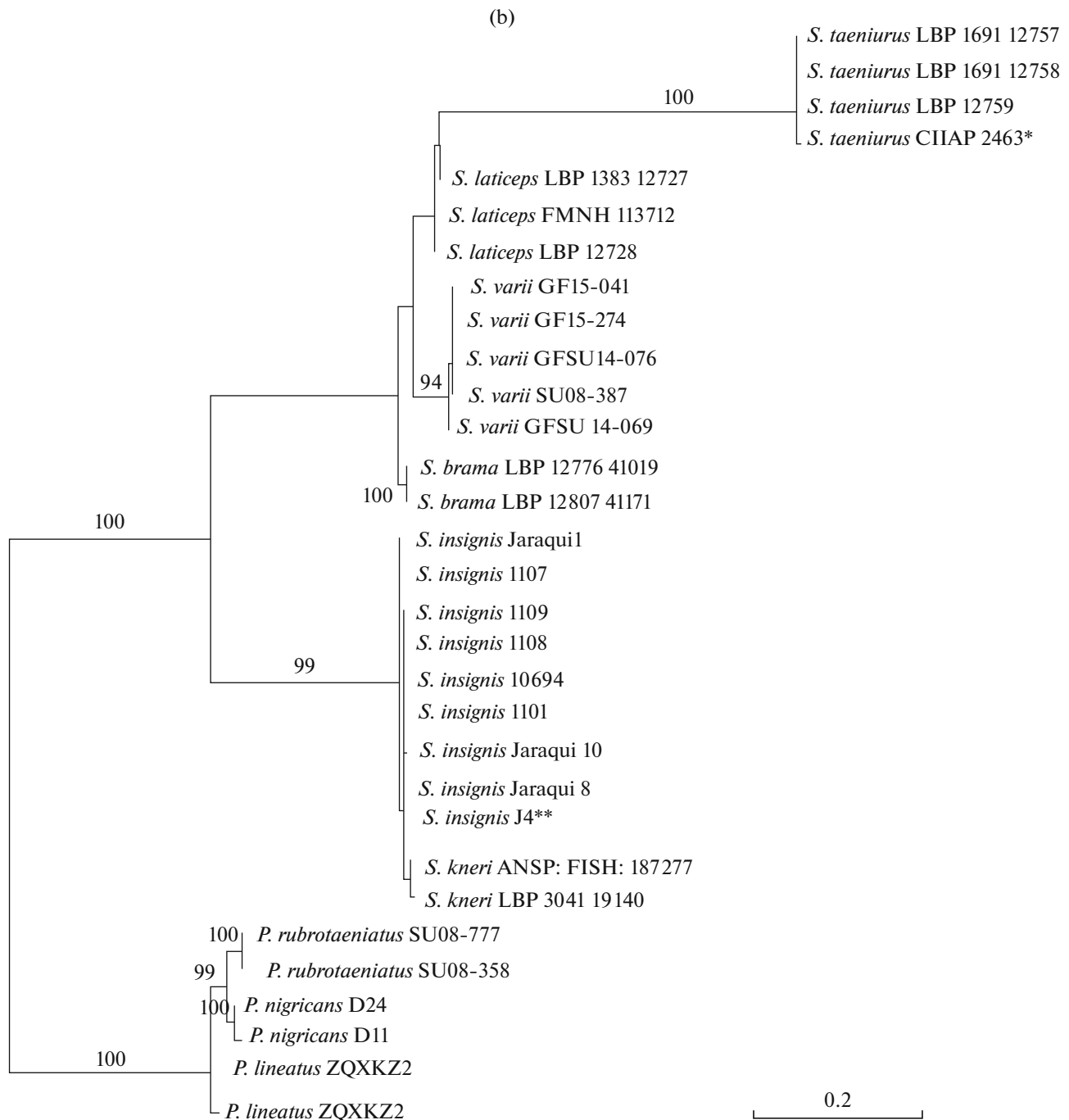


Fig. 3. (End.)

collected in Lizardo stream, which was 7 m wide and 4 m deep, featuring moderate current and blackwater with a light mixture of whitewater from the Putumayo River (Fig. 1). The stream had shrub and tree vegetation and an abundance of *Myrciaria dubia* (camu camu) plants. The water in the stream had a pH of 7.4, a temperature of 28.6°C, an electrical conductivity of 124.1 $\mu\text{S}/\text{cm}$, and total dissolved solids of 81.2 ppm.

Conservation Status

According to the IUCN database, five of the six species of *Semaprochilodus* are categorized as Least Concern, except for *S. insignis*, which has not been categorized to date. *S. insignis* is characterized by having an unknown type locality and a wide distribution throughout the Amazon basin. With the record of *S. taeniurus* in the Peruvian Amazon, the range of the

Table 2. Estimates of evolutionary divergence between sequences of *Semaprochilodus* spp.

no.	Name	Sp01	Sp02	Sp03	Sp04	Sp05	Sp06	Sp07	Sp08	Sp09	Sp10	Sp11	Sp12	Sp13	Sp14	Sp15	Sp16	Sp17	Sp18	Sp19	Sp20	Sp21	Sp22	Sp23	Sp24	Sp25
Sp01	<i>S. brama</i> LBP 12776-41019																									
Sp02	<i>S. brama</i> LBP 12807-41171	0																								
Sp03	<i>S. taeniurus</i> LBP 12759	13.0	13.0																							
Sp04	<i>S. laticeps</i> LBP 1383-12727	2.2	2.2	12.0																						
Sp05	<i>S. laticeps</i> LBP 12728	1.8	1.8	10.5	0.2																					
Sp06	<i>S. kneri</i> ANSP: FISH:187277	10.8	10.8	13.2	12.0	10.6																				
Sp07	<i>S. kneri</i> LBP 3041-19140	10.5	10.5	13.4	11.8	10.2	0.2																			
Sp08	<i>S. varii</i> GF15-274	2.8	2.8	12.2	3.0	2.5	11.2	11.5																		
Sp09	<i>S. laticeps</i> FMNH 113712	2.1	2.1	12.3	0.2	0.0	11.8	11.5	2.8																	
Sp10	<i>S. varii</i> GFSU14-076	2.8	2.8	12.2	3.0	2.5	11.2	11.5	0.0	2.8																
Sp11	<i>S. varii</i> GF15-041	2.8	2.8	12.2	3.0	2.5	11.2	11.5	0.0	2.8	0															
Sp12	<i>S. varii</i> GFSU14-069	2.6	2.6	12.5	2.8	2.5	11.5	11.7	0.2	2.6	0.2	0.2														
Sp13	<i>S. varii</i> SU08-387	2.9	2.9	11.8	2.9	2.6	12.3	12.6	0.0	2.9	0	0	0.2													
Sp14	<i>S. insignis</i> 1108	11.0	11.0	12.8	12.1	10.2	0.6	0.4	11.8	11.8	11.8	11.8	12.1	13.2												
Sp15	<i>S. insignis</i> 1101	11.0	11.0	12.8	12.1	10.2	0.6	0.4	11.8	11.8	11.8	11.8	12.1	13.2	0											
Sp16	<i>S. taeniurus</i> CIAP 2463*	12.7	12.7	0.2	11.9	10.8	12.6	12.8	12.2	12.2	12.2	12.2	12.4	11.7	12.5	12.5										
Sp17	<i>S. insignis</i> 1109	11.0	11.0	12.8	12.1	10.2	0.6	0.4	11.8	11.8	11.8	11.8	12.1	13.2	0	0	12.5									
Sp18	<i>S. insignis</i> 10694	11.0	11.0	12.8	12.1	10.2	0.6	0.4	11.8	11.8	11.8	11.8	12.1	13.2	0	0	12.5	0								
Sp19	<i>S. insignis</i> 1107	10.7	10.7	12.5	11.8	9.9	0.8	0.6	11.6	11.6	11.6	11.6	11.8	12.9	0.2	0.2	12.2	0.2	0.2							
Sp20	<i>S. taeniurus</i> LBP 1691-12758	13.0	13.0	0	12.0	10.5	13.2	13.4	12.2	12.3	12.2	12.2	12.5	11.8	12.8	12.8	0.2	12.8	12.8	12.5						
Sp21	<i>S. taeniurus</i> LBP 1691-12757	13.0	13.0	0	12.0	10.5	13.2	13.4	12.2	12.3	12.2	12.2	12.5	11.8	12.8	12.8	0.2	12.8	12.8	12.5	0					
Sp22	<i>S. insignis</i> J4**	10.5	10.5	13.2	11.8	10.2	0.5	0.3	11.5	11.5	11.5	11.5	11.7	12.6	0	0	12.6	0	0	0.2	13.2					
Sp23	<i>S. insignis</i> Jaraqui 10	10.8	10.8	13.4	12.2	11.0	0.7	0.5	11.8	11.9	11.8	11.8	12.1	12.3	0.2	0.2	12.8	0.2	0.2	0.4	13.4	13.4	0.2			
Sp24	<i>S. insignis</i> Jaraqui 8	11.1	11.1	13.1	12.4	11.0	0.5	0.3	12.1	12.2	12.1	12.1	12.4	12.6	0	0	12.5	0	0	0.2	13.1	13.1	0	0.2		
Sp25	<i>S. insignis</i> Jaraqui 1	10.8	10.8	12.8	12.2	10.6	0.7	0.5	11.8	11.9	11.8	11.8	12.1	12.3	0.2	0.2	12.2	0.2	0.2	0.0	12.8	12.8	0.2	0.3	0.2	

S. taeniurus* species sequenced in this study, *S. taeniurus* which should be corrected for *S. insignis*. Orange color indicates genetic distance between *S. insignis*. Green color indicates genetic distance between *S. taeniurus*.

Table 3. Morphometric and meristic data for *Semaprochilodus taeniurus* (the number of samples $n = 3$)

Morphometric and meristic data	Min	Max	Mean \pm SD
Standard length (<i>SL</i>), mm	189.1	194.2	191.6
	In % <i>SL</i>		
Greatest body depth	34.5	36.4	35.2 \pm 1.0
Predorsal length	44.4	45.1	44.7 \pm 0.4
TDorsal-fin base length	14.8	15.6	15.3 \pm 0.4
Dorsal fin to adipose fin distance	27.5	28.0	27.8 \pm 0.3
Dorsal fin to caudal fin distance	43.2	45.5	44.4 \pm 1.2
Prepelvic length	49.3	51.1	50.3 \pm 0.9
Preanal distance	74.9	75.7	75.2 \pm 0.5
Snout to anal-fin insertion	79.5	80.7	80.2 \pm 0.6
Anal-fin base length	9.2	9.7	9.4 \pm 0.2
Caudal-peduncle length	13.9	14.9	14.4 \pm 0.5
Dorsal-fin length	27.8	29.9	28.7 \pm 1.1
Pectoral-fin length	18.1	18.6	18.3 \pm 0.3
Pelvic-fin length	18.8	19.7	19.1 \pm 0.5
Least caudal-peduncle height	9.9	10.4	10.2 \pm 0.3
Head length (<i>HL</i>)	26.9	27.9	27.3 \pm 0.5
	In % <i>HL</i>		
Snout length	36.3	36.7	36.6 \pm 0.2
Bony orbital diameter	28.7	30.5	29.7 \pm 0.9
Postorbital length	37.9	41.8	40.3 \pm 2.1
Interorbital width	49.0	52.2	50.8 \pm 1.6
Mouth width	37.4	40.5	38.8 \pm 1.5
	Counts		
Lateral-line scales	68	70	69
Scale rows between dorsal-fin origin and lateral line	12	13	13
Scale rows between anal-fin origin and lateral line	9	10	10
Scale rows between pelvic-fin insertion and lateral line	13	13	13
Rows of scales around caudal peduncle	24	25	24
Median predorsal scales	18	20	19
Median scales between dorsal and adipose fins	19	21	20
Inner row teeth, upper jaw	10	11	10
Inner row teeth, lower jaw	6	7	7

species exceeds 20000 km². Although this species appears to be rare in the Peruvian Amazon and is currently only known in the Putumayo basin, it is necessary to carefully review the fishing records, as Peru had previously considered only the presence of *S. insignis*.

DISCUSSION

A comprehensive understanding of the ichthyofauna in the Peruvian continental environments is of great importance for the rational and successful planning of an ecosystem management program. It is known that Amazonian ecosystems harbor a high

diversity of fish, and the Peruvian Amazon basin stands out as the type locality for over 500 fish species. Many similar studies have shown that DNA barcodes are becoming an increasingly prominent tool in the identification and description of new species, as well as in the development of biodiversity inventories and species catalogs (García-Davila et al., 2018, 2021; Chuctaya et al., 2021, 2023). Recent studies have highlighted the value of combining morphological and molecular data in taxonomy (Ramirez et al., 2017; Chuctaya et al., 2020). In the present study, we identified *Semaprochilodus taeniurus* species in the Putumayo basin for the first time, confirmed by morphologi-

cal characteristics and DNA barcodes. The morphological characteristics of *S. taeniurus* were consistent with those previously reported by Castro and Vari (2004), indicating a relatively conservative morphology.

Castro and Vari (2004) indicates that of the six valid species in the genus *Semaprochilodus*, *S. taeniurus* differs from *S. brama*, *S. laticeps*, and *S. varii* by its lack of intense dark pigmentation on the opercular membranous border and the exposed surface of the pectoral fin. In the case of *S. insignis* and *S. kneri*, these species share the same morphological characteristic mentioned above, along with *S. taeniurus*. However, *S. taeniurus* can be differentiated from *S. insignis* (with which it is found in syntopy) and *S. kneri* based on meristic characteristics. *S. taeniurus* exhibits a higher number of scales in the lateral line, above the pelvic fin and the lateral line, and around the caudal peduncle (Table 3). Our results regarding the external, morphometric, and meristic characteristics of the three specimens analyzed are consistent and corroborate what was mentioned by Castro and Vari (2004), validating the identification of *S. taeniurus*.

In the genetic analysis, our sequences showed a 99.8% identity with *S. taeniurus* when used on NCBI. Currently, there were four sequences of *S. taeniurus* available in GenBank from the middle part of the Amazon basin (Manaus), including three sequences (KX086751, KX086752, KX086753) from Melo et al. (2016) and one sequence (FJ457765) from the sampling of commercially important fishes in the Brazilian Amazon by Ardura et al. (2010). Based on our analyses, the *Semaprochilodus* species collected in the Putumayo River basin show a genetic distance of 0.18% from the species reported by Melo et al. (2016), while it differs by 11.32% from the species recorded by Ardura et al. (2010), indicating that the species sequenced by Ardura et al. (2010) would be *S. insignis*, with which it shares a genetic distance of 0 to 0.19%. In the phylogenetic analysis using the *COI* gene, all species formed distinct group in the trees. The *Semaprochilodus* samples from the Putumayo clustered with *S. taeniurus* from the middle Amazon basins, Manaus, Brazil, forming a strongly supported clade with a bootstrap value of 1.00.

In recent years, various ichthyological inventories have been conducted along the Putumayo River and its main tributaries (Hidalgo and Oliveira, 2004; Ortega et al., 2006; Hidalgo and Ortega-Lara, 2011; Hidalgo and Maldonado-Ocampo, 2016; Faustino-Fuster et al., 2021). In addition, we can add the official lists of fish species provided by Ortega et al. (2012) and Meza-Vargas et al. (2021). All the published references do not record *Semaprochilodus taeniurus* as a valid species for Peru. The few references that mention the presence of this species are cited by Melo et al. (2016), who conducted the first molecular phylogeny of the Prochilodontidae family. Melo et al. (2016) analyzed specimens of *S. taeniurus* (LBP 1691) from the Ama-

zon basin in the Manaus region, Brazil, which is the type locality of the species in question. In Colombia, this species was documented by Correa (2003) in Lake Taraira, a part of the Apoporis River basin, which is a blackwater tributary of the Caquetá-Japurá River. The collection involved a single specimen (ICN-MHN-4331). This sole record was later referenced by DoNascimento et al. (2017), leading to the inclusion of this species in the list of fishes for Colombia. These limited records categorize this species as rare in the upper part of the Amazon basin.

In Perú, Meza-Vargas et al. (2021) only reported the presence of *Semaprochilodus insignis* as the sole representative of that genus in Peru. It is likely that the timing and location of the sampling, as well as the fishing techniques used, did not allow for the record of *S. taeniurus*. A similar situation may have occurred in other river basins in the Peruvian Amazon (Ortega et al., 2003; Hidalgo and Willink, 2007; Correa and Ortega, 2010; Sánchez et al., 2013; Corahua et al., 2015; Chuctaya et al., 2022).

Additionally, recent field research has reported several new fish species and new records for the upper part of the Amazon basin using an integrative morphological and molecular analysis, highlighting the potential of this methodology to expedite the species identification process. This underscores the Upper Amazon basin's significance in hosting a high ichthyological diversity (Chuctaya et al., 2021, 2023). Furthermore, the presence of *Semaprochilodus taeniurus* could also be explained by its migratory capacity related to its reproductive, trophic, and ecological aspects, allowing them to cover hundreds of kilometers between river basins using the main channel of the Amazon (Goulding et al., 1988; Ribeiro and Petreire, 1990; Sivansudar et al., 2001; Duponchelle et al., 2021).

Therefore, our morphological and molecular results presented in this study provide formal evidence of the presence of *Semaprochilodus taeniurus* in aquatic environments in the Peruvian Amazon based on specimens collected from the Putumayo River. This highlights the importance of continuing to conduct ichthyological inventories, especially in areas with information gaps. These efforts contribute to enriching the ichthyofauna of the country and enhancing our understanding of species distribution patterns in the Amazon River basin.

CONCLUSIONS

Three specimens of *Semaprochilodus taeniurus* are preserved in the ichthyological collection of CIAP. The occurrence of this species in the Putumayo River basin, which serves as a border between Peru and Colombia, signifies an extension of the distribution of *S. taeniurus* in Colombia and the first record for Peru. The study also highlights that by using integrative methods, more species, as well as new records and new

species for the Peruvian Amazon basin, could be discovered. Additionally, this information enriches the molecular database of Peruvian species, which will serve as data for environmental DNA studies.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1134/S0032945224700206>.

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AUTHOR CONTRIBUTION

M. Ruiz-Tafur was responsible for the conceptualization, data curation, methodology, research, writing-draft preparation, writing (review and editing), supervision. J. R. Garcia-Ayala: conceptualization, methodology, research and writing (review and editing). C. Angulo, D. Castro-Ruiz: methodology, research, data curation, writing (review and editing). C. Garcia-Davila: writing (review and editing). J. Chuctaya: data curation, methodology, research, writing-draft preparation, writing (review and editing). All authors have read and approve the final version of the manuscript.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The authors declare not to have violated or omitted ethical or legal standards when conducting the research and creating this manuscript. This study has a collection permit granted by the Regional Directorate of Production Loreto (DIREPRO-L), with No. 598-2022-GRL/DIREPRO.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

REFERENCES

- Ardura, A., Linde, A.R., Moreira, J.C., and Garcia-Vazquez, E., DNA barcoding for conservation and management of Amazonian commercial fish, *Biol. Conserv.*, 2010, vol. 143, pp. 1438–1443.
<https://doi.org/10.1016/j.biocon.2010.03.019>
- Ardura, A., Planes, S., and Garcia-Vazquez, E., Applications of DNA barcoding to fish landings: authentication and diversity assessment, *ZooKeys*, 2013, vol. 365, pp. 49–65.
<https://doi.org/10.3897/zookeys.365.6409>
- Castro, R.M.C. and Vari, R.P., Family Prochilodontidae, in *Check List of the Freshwater Fishes of South and Central America*, Reis, R.E., Kullander, S.O., and Ferraris, C.J., Eds., Porto Alegre: Edipucrs, 2003, pp. 65–70
- Castro, R.M.C. and Vari, R.P., *Detritivores of the South American Fish Family Prochilodontidae (Teleostei: Ostariophysi: Characiformes): A Phylogenetic and Revisionary Study*, Washington: Smithsonian Contributions to Zoology, 2004.
- Chen, W., Li, C., Yang, J., et al., Temporal species-level composition of larvae resources in the lower Pearl River drainage and implications for species' reproductive cycles, *Gene*, 2021, vol. 776, Article 145351.
<https://doi.org/10.1016/j.gene.2020.145351>
- Chuctaya, J., Ohara, W.M., and Malabarba, L.R., A new species of *Odontostilbe* cope (Characiformes: Cheirodontinae) from rio Madeira basin diagnosed based on morphological and molecular data, *J. Fish Biol.*, 2020, vol. 97, pp. 1701–1712.
<https://doi.org/10.1111/jfb.14533>
- Chuctaya, J., Encalada, A.C., Barragán, K.S., et al., New ecuadorian records of the eyeless banjo catfish *Micromyzon akamai* (Siluriformes: Aspredinidae) expand the species range and reveal intraspecific morphological variation, *J. Fish Biol.*, 2021, vol. 98, pp. 1186–1191.
<https://doi.org/10.1111/jfb.14630>
- Chuctaya, J., Meza-Vargas, V., Faustino-Fuster, D.R., et al., Lista de especies de peces de la cuenca del río Ucayali, Perú, *Revista Peruana de Biología*, 2022, vol. 29, pp. 1–55.
<https://doi.org/10.15381/rpb.v28iespecial.21911>
- Chuctaya, J., Shibatta, O.A., Encalada, A.C., et al., Rediscovery of *Rhyacoglanis pulcher* (Boulenger, 1887) (Siluriformes: Pseudopimelodidae), a rare rheophilic bumblebee catfish from Ecuadorian Amazon, *PLoS One*, 2023, vol. 18, pp. 1–16.
<https://doi.org/10.1371/journal.pone.0287120>
- Corahua, I., Aldea-Guevara, M.I., and Hidalgo, M.H., *Fishes, in Perú: Tapiche-Blanco. Rapid Biological and Social Inventories Report 27*, Pitman, N. et al., Eds., and Chicago: Field Museum, 2015, pp 109–117
- Correa, S. B., Ichthyofauna of lago Taraira, lower río Apaporis system, Colombian Amazon. *Dahlia*, 2003, vol. 6, pp. 59–68.
<https://doi.org/10.15381/rpb.v17i1.48>
- Correa, E. and Ortega, H., Diversidad y variación estacional de peces en la cuenca baja del río Nanay, Perú, *Revista Peruana de Biología*, 2010, vol. 17, pp. 37–42.
<https://doi.org/10.15381/rpb.v17i1.48>

- Cutarelli, A., Amoroso, M. G., De Roma, A., et al., Italian market fish species identification and commercial frauds revealing by DNA sequencing, *Food Control*, 2014, vol. 37, pp. 46–50.
- Dagosta, F.C. and De Pinna, M., The fishes of the Amazon: Distribution and biogeographical patterns, with a comprehensive list of species, *Bull. Am. Mus. Nat. History*, 2019, vol. 431, pp. 1–163.
<https://doi.org/10.1206/0003-0090.431.1.1>
- Da Silva, F.A., Feldberg, E., Carvalho, N.D.M., et al., Effects of environmental pollution on the rDNAomics of Amazonian fish, *Environ. Pollut.*, 2019, vol. 252, pp. 180–187.
<https://doi.org/10.1016/j.envpol.2019.05.112>
- DoNascimento, C., Herrera-Collazos, E.E., Herrera, G.A., et al., Checklist of the freshwater fishes of Colombia: A Darwin core alternative to the updating problem, *ZooKeys*, 2017, vol. 708, pp. 25–138.
<https://doi.org/10.3897/zookeys.708.13897>
- Doyle, J.J. and Doyle, J.L., A rapid DNA isolation procedure from small quantities of fresh leaf tissue, *Phytochem. Bull.*, 1987, vol. 9, pp. 11–15.
- Duponchelle, F., Isaac, V.J., Rodrigues, D.C., et al., Conservation of migratory fishes in the Amazon basin, *Aquatic Conserv.: Mar. Freshw. Ecosyst.*, 2021, vol. 31, pp. 1087–1105.
<https://doi.org/10.1002/aqc.3550>
- Faustino-Fuster, D.R., Patarroyo Báez, J.J., and de Souza, L.S., Fishes, in *Colombia, Perú: Bajo Putumayo-Yaguas-Cotuhé. Rapid Biological and Social Inventories Report 31*, Jarrett, C.C., Eds., Chicago: Field Museum, 2021, pp. 138–145
- Fricke, R., Eschmeyer, W.N., and Van der Laan, R., *Eschmeyer's Catalog of Fishes: Genera, Species, References, Version 10/2023*, 2023. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>.
- García-Dávila, C., Sánchez, H., Flores, M., et al., *Peces de consumo de la Amazonía peruana*, Perú: Instituto de Investigaciones de la Amazonía Peruana, 2018.
- García-Dávila, C., Estivals, G., Mejia, J., et al., *Peces ornamentales de la Amazonía peruana*, Perú: Instituto de Investigaciones de la Amazonía Peruana, 2020.
- Goulding, M., Carvalho, M.L., and Ferreira, E.G., *Rio Negro: Rich Life in Poor Water*, Hague: SPD Academica Publ., 1988.
- Goulding, M., Venticinque, E., Ribeiro, M.L.D.B., et al., Ecosystem-based management of Amazon fisheries and wetlands, *Fish Fisheries*, 2018, vol. 20, pp. 138–158.
<https://doi.org/10.1111/faf.12328>
- Guimarães, K.L., Rosso, J.J., Souza, M.F., et al., Integrative taxonomy reveals disjunct distribution and first record of *Hoplias misionera* (Characiformes: Erythrinidae) in the Amazon River basin: Morphological, DNA barcoding and cytogenetic considerations, *Neotrop. Ichthyol.*, 2021, vol. 19, Article e200110.
<https://doi.org/10.1590/1982-0224-2020-0110>
- Hall, T.A., BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucl. Acids Symp Ser.*, 1999, vol. 41, pp. 95–98.
https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29
- Hebert, P.D., Ratnasingham, S., and De Waard, J.R., Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species, *Proc. Royal Soc. London. Ser. B, Biol. Sci.*, 2003, vol. 270, pp. 96–99.
<https://doi.org/10.1098/rsbl.2003.0025>
- Hidalgo, M., and Maldonado-Ocampo, J., Fishes, in *Perú: Medio Putumayo-Algodón. Rapid Biological and Social Inventories Report 28*, Pitman, N., Eds., Chicago: Field Museum, 2016, pp. 109–119.
- Hidalgo, M., and Olivera, R., Fishes, in *Perú: Ampiyacu, Apayacu, Yaguas, Medio Putumayo. Rapid Biological Inventories Report 12*, Pitman, N., Eds., Chicago: Field Museum, 2004, pp. 62–67.
- Hidalgo, M. and Ortega-Lara, A., Fishes, in *Perú: Yaguas-Cotuhé. Rapid Biological and Social Inventories Report 23*, Pitman, N., Eds., Chicago: Field Museum, 2011, pp. 98–107.
- Hidalgo, M. and Willink, P.W., Fishes, in *Perú: Nanay-Mazán-Arabela. Rapid Biological Inventories Report 18*, Vriesendorp, C., Eds., Chicago: Field Museum, 2007, pp. 125–130.
- Lopes, U., Galetti, P.M., Jr., and Domingues de Freitas, P., Hidden diversity in *Prochilodus nigricans*: A new genetic lineage within the Tapajós River basin, *LloS One*, 2020, vol. 15, no. 8, Article e0237916.
<https://doi.org/10.1371/journal.pone.0237916>
- Mariac, C., Renno, J.F., Vigouroux, Y., et al., Species-level ichthyoplankton dynamics for 97 fishes in two major river basins of the Amazon using quantitative metabarcoding, *Mol. Ecol.*, 2022, vol. 31, no. 6, pp. 1627–1648.
- Martins, T., Santana, P., Lutz, Í., et al., Intensive commercialization of endangered sharks and rays (Elasmobranchii) along the coastal Amazon as revealed by DNA barcode, *Front. Mar. Sci.*, 2021, vol. 8, Article 769908.
<https://doi.org/10.3389/fmars.2021.769908>
- Melo, B.F. and Sidlauskas, B.L., Family Prochilodontidae—Flannel mouth characiforms, in *Field Guide to the Fishes of the Amazon, Orinoco, and Guianas*, Van der Sleen, P., Albert, J.S., Eds., Princeton Univ. Press, 2017, pp. 170–172.
- Melo, B.F., Sidlauskas, B.L., Hoekzema, K., et al., The first molecular phylogeny of Chilodontidae (Teleostei: Ostariophysi: Characiformes) reveals cryptic biodiversity and taxonomic uncertainty, *Mol. Phylogen. Evol.*, 2014, vol. 70, pp. 286–295.
<https://doi.org/10.1016/j.ympev.2013.09.025>
- Melo, B.F., Sidlauskas, B.L., Hoekzema, K., et al., Molecular phylogenetics of the Neotropical fish family Prochilodontidae (Teleostei: Characiformes), *Ibid.*, 2016, vol. 102, pp. 189–201.
<https://doi.org/10.1016/j.ympev.2016.05.037>
- Meza-Vargas, V., Faustino-Fuster, D.R., Chuctaya, J., et al., Checklist of freshwater fishes from Loreto, Perú, *Revista Peruana de Biología*, 2021, vol. 28, pp. 1–28.
<https://doi.org/10.15381/rpb.v28iespecial.21911>
- Miller, S.E., DNA barcoding and the renaissance of taxonomy, *Proc. Natl. Acad. Sci.*, 2007, vol. 104, no. 12, pp. 4775–4776.
- Miller, M.A., Pfeiffer, W., and Schwartz, T., Creating the CIPRES science gateway for inference of large phylogenetic trees, in *2010 Gateway Computing Environments Workshop (GCE)*, New Orleans, 2010, pp. 1–8.
<https://doi.org/10.1109/GCE.2010.5676129>
- Miranda-Chumacero, G., Mariac, C., Duponchelle, F., et al., Threatened fish spawning area revealed by specific metabarcoding identification of eggs and larvae in the Beni

- River, upper Amazon, *Global Ecol. Conserv.*, 2020, vol. 24, Article e01309.
<https://doi.org/10.1016/j.gecco.2020.e01309>
- Nei, M. and Kumar, S., *Molecular Evolution and Phylogenetics*, New York: Oxford Univ. Press, 2000.
- Ortega, H., Hidalgo, M., and Bértiz, G., The Fish of the Yavari River, in *Yavari: Rapid Biological Inventories Report 11*, Pitman, N., Eds., Chicago: Field Museum, 2003, pp. 220–243
- Ortega, H., Mojica, J.I., Alonso, J.C., and Hidalgo, M., Listado de los peces de la cuenca del río Putumayo en su sector colombo-peruano, *Biota Colombiana*, 2006, vol. 7, pp. 95–111.
- Ortega, H., Hidalgo, M., Trevejo, G., et al., *Lista anotada de los peces de aguas continentales del Perú: Estado actual del conocimiento, distribución, usos y aspectos de conservación*, Perú: Ministerio del Ambiente; Dirección General de Diversidad Biológica, 2012.
- Ota, R.P., Machado, V.N., Andrade, M.C., et al., Integrative taxonomy reveals a new species of pacu (Characiformes: Serrasalminidae: Myloplus) from the Brazilian Amazon, *Neotrop. Ichthyol.*, 2020, vol. 18, no. 1, Article e190112.
<https://doi.org/10.1590/1982-0224-20190112>
- Queiroz, L.J., Ohara, W.M., and Vari, R.P., Prochilodontidae, in *Peixes do Rio Madeira*, São Paulo: Dialetto, 2013, pp. 140–145.
- Papa, Y., Le Bail, P.Y., and Covain, R., Genetic landscape clustering of a large DNA barcoding data set reveals shared patterns of genetic divergence among freshwater fishes of the Maroni Basin, *Mol. Ecol. Res.*, 2021, vol. 21, pp. 2109–2124.
<https://doi.org/10.1111/1755-0998.13402>
- Ramirez, J.L., Birindelli, J., Carvalho, D.C., et al., Revealing hidden diversity of the underestimated neotropical ichthyofauna: DNA barcoding in the recently described genus Megaleporinus (Characiformes: Anostomidae), *Front. Genet.*, 2017, vol. 8, pp. 1–11.
<https://doi.org/10.3389/fgene.2017.00149>
- Ratnasingham, S., and Hebert, P. D., BOLD: The barcode of life data system (<http://www.barcodinglife.org>), *Mol. Ecol. Not.*, 2007, vol. 7, no. 3, pp. 355–364.
<https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Ribeiro, M.C.L.B. and Petrere M., Jr., Fisheries ecology and management of the jaraqui (*Semaprochilodus taeniurus*, *S. insignis*) in Central Amazonia, *Regul. Rivers: Res. Manag.*, 1990, vol. 5, pp. 195–215.
<https://doi.org/10.1002/rrr.3450050302>
- Sánchez, H., Nolorbe, C., García, A., et al., Diversidad y abundancia de peces en los ríos Arabela y Curaray (cuenca del río Napo) en época de creciente y vaciante del 2012, amazonia peruana, *Folia Amazónica*, 2013, vol. 22, pp. 43–58.
<https://doi.org/10.24841/fa.v22i1-2.47>
- Schindel, D. and Miller, S., DNA barcoding a useful tool for taxonomists, *Nature*, 2005, vol. 435, Article 17.
<https://doi.org/10.1038/435017b>
- Silvano, R.A.M., Nitschke, P.P., Vieira, K.C., et al., Atlas of fish of Tapajós and Negro Rivers I: Characiformes, in *Fish and Fisheries in the Brazilian Amazon. People, Ecology and Conservation in Black and Clear Water Rivers*, Silvano, R.A.M., Ed., Porto Alegre: Springer Internat. Publ., 2020, pp. 41–196.
- Sivasundar, A., Bermingham, E., and Ortí, G., Population structure and biogeography of migratory freshwater fishes (Prochilodus: Characiformes) in major South American rivers, *Mol. Ecol.*, 2001, vol. 10, pp. 407–417.
<https://doi.org/10.1046/j.1365-294X.2001.01194.x>
- Souza, C.S., Mattox, G.M., Vita, G., et al., Molecular species delimitation and description of a new species of Phenacogaster (Teleostei, Characidae) from the southern Amazon basin, *ZooKeys*, 2023, no. 1164, pp. 1–21.
<https://doi.org/10.3897/zookeys.1164.102436>
- Stamatakis, A., RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models, *Bioinformatics*, 2006, vol. 22, pp. 2688–2690.
<https://doi.org/10.1093/bioinformatics/btl446>
- Stamatakis, A., Hoover, P., and Rougemont, J., A., Rapid bootstrap algorithm for the RAxML Web servers, *Systemat. Biol.*, 2008, vol. 57, pp. 758–771.
<https://doi.org/10.1080/10635150802429642>
- Tamura, K., Stecher, G., and Kumar, S., MEGA11: Molecular evolutionary genetics analysis version 11, *Mol. Biol. Evol.*, 2021, vol. 38, pp. 3022–3027.

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