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Is there hidden genetic variability in the genus *Steindachneridion* Garavello, 2005 (Siluriformes: Pimelodidae)?

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Background: Neotropical fish species belonging to the *Steindachneridion* genus are frequently categorized as endangered due to their high level of endemism. **Methods:** In this study, genetic analyses of mitochondrial DNA (mtDNA) D-loop were conducted on four species within this genus across their respective distributions: *Steindachneridion scriptum* (from the Tibagi and Uruguay Rivers), *S. melanodermatum* (from the Iguaçu River), *S. doceanum* (from the Doce River), and *S. parahybae* (from the Paraíba do Sul River). *Zungaro zungaro* and *Brachyplatystoma rousseauxii* were employed as outgroups, and the topology was inferred using Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic reconstruction techniques. Sequences were also analyzed to assess genetic diversity levels. **Results:** Contrary to the remaining species, which exhibited distinct species-specific clades, our data suggests that *S. scriptum* formed two sister clades, potentially representing distinct operational taxonomic units. Novel haplotypes were identified for each of the four species, further supporting the conclusions derived from the phylogenetic analysis. Overall, *Steindachneridion* species displayed high haplotype diversity paired with low nucleotide diversity, indicating a demographic expansion event after a period of reduced effective population size. Nevertheless, genetic structure indexes were notably high. These findings imply that the genetic diversity within these species might be undervalued, holding implications for both taxonomic classification and biological conservation strategies. **Conclusion:** In conclusion, the study of genetic diversity in *Steindachneridion* species has revealed distinct Molecular Operational Taxonomic Units (MOTUs), highlighting the need for conservation efforts. The detection of new haplotypes

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ABSTRACT

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Conclusion: In conclusion, the study of genetic diversity in *Steindachneridion* species has revealed distinct Molecular Operational Taxonomic Units (MOTUs), highlighting the need for conservation efforts. The detection of new haplotypes and intraspecific variability emphasizes the urgency for systematic conservation measures in the face of looming extinction threats.

INTRODUCTION

Steindachneridion is a fish genus endemic to the eastern Brazilian watersheds and can also be found in the rivers of the Paraná and Uruguay basins (*Garavello, 2005*). Although its placement within the Pimelodidae is well accepted, its evolutionary history and ancestry remain largely unknown (*Albert & Reis, 2011*). It is regarded as a basal group for the superfamily Pimelodoidea despite its unresolved phylogenetic relationships (*Sullivan et al., 2013*).

In terms of taxonomy, six species are recognized under the genus *Steindachneridion* (*Garavello, 2005*). *Steindachenridion* fossils of the *S. iheringi* species were reported by *Woodward, 1898* and dated to the Oligocene (*Lima, 1985*), indicating that they first emerged around 13.5 million years ago, which is compatible with the diversification of pimelodid fish.

The existence of a hidden diversity in this genus is questioned by some studies using molecular markers, although morphological taxonomy recognizes some interspecific diversity in *Steindachneridion* (*Matoso, 2009; Matoso et al., 2011; Paixão et al., 2018*). For *S. parahybae*, a species on the verge of extinction (*Honji et al., 2017*), the use of microsatellite markers and gene sequences from the mitochondrial DNA controlling region indicated population structure due to fragmentation and anthropic impacts. However, without showing any signs of population organization, an unrooted haplotype network analysis employing *D-loop* sequences separately grouped the species *S. scriptum*, *S. parahybae*, and *S. melanodermatum* (*Souza-Shibatta et al., 2021*).

According to ICMBio/MMA (*ICMBio, 2014*), *Steindachneridion* species frequently appear at the top of Red Book lists of endangered species. This is due to a number of urgent issues, including overfishing and habitat degradation and fragmentation, which are considered to be the primary causes of the decline and extinction of freshwater fish species (*Paixão et al., 2018*). Given this situation, defining significant taxonomic units for conservation as well as mapping population structure and genetic variability are crucial sources of information that serve as a basis for formulating effective management strategies in the restructuring and/or maintenance of populations and species of *Steindachneridion*. To better understand the phylogenetic and demographic relationships of this important group of Neotropical fish, the goal of this paper is to present fresh information concerning molecular genetic variability.

MATERIALS & METHODS

Sampling and D-loop PCR

Muscle samples were collected for the DNA assessment: ten *Steindachneridion scriptum* specimens from Rio Uruguay, six from Rio Tibagi, seventeen *S. melanodermatum* specimens from Rio Iguaçu, three *S. parahybae* specimens from Rio Paraíba do Sul, and two *S. doceanum* specimens from Rio Doce (Figure 1), following the procedures outlined by (*Sambrook et al., 1989*).

----- Figure 1 here -----

The amplification of the D-loop region was conducted using 5 ng of DNA, 1.5 mM MgCl2, 1x buffer, 200 mM of each dNTP, 0.5 U of Taq DNA polymerase, and 0.5 mM of each primer (FTTF: 5'CAA AGC GCC GGT CTT 3 ' and F12R 5'GTC AGG ACC ATG CCT TTG3') (*Sivasundar et al., 2001*). PCR was initiated with five cycles, comprising denaturation at 94ºC for one minute, hybridization at 53ºC for one minute, and elongation at 72ºC for one minute and 30 seconds, followed by 25 cycles at 50ºC for the hybridization step. PCR reactions were conducted using a PTC-100 thermocycler from MJ Research. PCR products were subjected to electrophoresis in a 0.8% agarose gel utilizing phage l as the molecular mass marker. Purification of samples was performed using the GFX PCR DNA and Gel Band Purification Kit (GE HealthCare), followed by sequencing using the DYEnamic ET Terminator Cycle Sequencing kit (GE HealthCare) as per the manufacturer's instructions. Bidirectional sequencing of all templates was carried out on a Sanger ABI 3130 sequencer (Applied Biosystems), with images documented by the Kodak Electrophoresis Analysis and Documentation System (EDAS) 290. Sequences of *Steindachneridion scriptum* from Rio Uruguay and Rio Tibagi (accession numbers EU930030-EU930044), *S. melanodermatum* from Rio Iguaçu (accession numbers OQ842480-OQ842496), *S. parahybae* from Rio Paraíba do Sul (accession numbers OQ842477-OQ842479), and *S. doceanum from* Rio Doce (accession numbers OQ842475-OQ84476) were deposited on GenBank.

This research followed the international standards of animal experimentation, approved by the Ethics Committee for Animal Use of the Universidade Estadual de Ponta Grossa (CEUA process number 0769342/2021). The collection was authorized by the Ministério do Meio Ambiente (MMA/ICMbio number 15115-1).

Genetic Diversity Analysis

Genetic distances within and between groups were estimated based on the Kimura 2-parameter evolution model (K2P) (*Kimura, 1980*). A *Neighbor-Joining* (NJ) tree (*Saitou & Nei, 1987*) was constructed from this model to graphically represent divergences between species to depict species divergences, and clade support was assessed by 1000 bootstrap pseudo-replicates according to (*Felsenstein, 1985*), using MEGA 10.2.2 (*Kumar et al., 2018*). Genetic diversity parameters (*S* - polymorphic sites; *Nh* - number of haplotypes; *h* - haplotypic diversity; π - nucleotide diversity) were evaluated utilizing DnaSP 6.12.03 (*Rozas et al., 2017*) and employed neutrality tests including *Tajima's* D (*Tajima, 1989*), *Fu's* Fs (*Fu, 1997*) and R_2 (*Ramos-Onsins & Rozas, 2002*).

Statistical significance was determined via 10,000 permutations under the coalescing process implemented in DnaSP 6.12.03 (*Rozas et al., 2017*). To elucidate relationships between species of the genus *Steindachneridion* and the population of *Steindachneridion melanodermatum* studied, haplotype networks were reconstructed using PopART 1.7 (*Leight & Bryant, 2015*) through the TSC method (*Clement et al., 2000*).

Phylogenetic Analysis of mtDNA D-loop Sequences

For the phylogenetic analyses, we retrieved a D-loop sequence from *Brachyplatystoma rousseauxii* with accession number DQ779046 (*Batista & Alves-Gomes, 2006*) and another sequence from *Zungaro zungaro* (accession number EU930046.1), as an outgroup from Genbank. These *D-loop* sequences underwent alignment and editing using the Clustal W program (*Thompson et al., 1994*). Subsequently, a Bayesian Inference (BI) analysis was conducted employing MrBayes v. 3.2.7 (*Ronquist & Huelsenbeck, 2003*) through Markov chain Monte Carlo (MCMC) searches on two simultaneous runs of four chains for 10^6 generations, sampling trees every 10^3 generations. A burn-in of 25% of the sampled trees was implemented, and consensus topology and nodal support were computed from the remaining trees, estimated as posterior probability values (*Huelsenbeck, 2001*). Visualization and finalization of phylogenetic trees were executed using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Additionally, the PAUP* program (*Swofford, 2002*) was utilized to determine the best evolutionary model via Modeltest 3.7 (*Posada & Crandall, 1998*) under the Hierarchical Likelihod Ratio Tests (hLRTs). The Maximum Likelihood (ML) analysis was performed using MEGA 11.0.13 (*Tamura et al., 2007*), employing the HKY model with a gamma distribution, and bootstrap resampling with 501 replications to assess node support.

RESULTS

Genetic Diversity of *Staindachneridion*

Nucleotide sequencing retrieved a total of 38 partial *D-loop* sequences of *Steindachneridion scriptum* samples collected from the Rio Uruguay and Rio Tibagi. The calculated average nucleotide diversity across these sequences was 0.065, and *S. scriptum* specifically exhibiting a nucleotide diversity of 0.017. Comparing of the nucleotide diversity and haplotype count within *S. scriptum* populations revealed that the Uruguay River harbored a greater number of haplotypes (N=10), whereas the Tibagi River displayed higher nucleotide diversity (N=6).

For *S. doceanum*, the determined nucleotide diversity was 0.030, while *S. melanodermatun* exhibited a nucleotide diversity of 0.011. Notably, a nucleotide diversity of 0.130 was observed in *S. parahybae*. The calculated R_2 values were substantially higher and statistically significant, indicating evidence of a departure from neutrality. However, in contrast, the Fu's (*Fs*) test metric showed relatively high values, which, despite their magnitude, did not achieve statistically significance.

It's important to note that the R_2 test has been acknowledged as more effective in detecting demographic events in smaller samples sizes (*Ramos-Onsins & Rozas, 2002*), as observed in the

present study. Conversely, Fu's (*Fs*) test tends to be more suitable for samples with a larger number of individuals, as emphasized in the comparison presented in Table 1.

----- Table 1 here -----

In the context of haplotype composition, our analysis revealed the identification of novel haplotypes within different species. Specifically, two new haplotypes (Haplo 1 and 2) were identified in *S. doceanum*, while three distinct haplotypes (Haplo37, Haplo38, and Haplo39) were found in *S. parahybae*. Moreover, a total of fifteen haplotypes were detected in *S. scriptum*, and sixteen haplotypes were observed in *S. melanodermatum*, labeled as Haplo28 to Haplo44 (as depicted in Figures 2–3).

----- Figure 2 here -----

----- Figure 3 here ----

In the haplotype network analysis, a closer relationship was observed between *S. scriptum* populations from Rio Tibagi and Rio Uruguay, with only 9 mutational steps separating these two populations. Comparatively, when contrasting *S. melanodermatum* with *S. scriptum* populations from the Uruguay River and Tibagi River, 13 and 18 mutational steps were identified, respectively. In addition, a considerable distance was found between *S. parahybae* and the other species, with 72 mutational steps from *S. parahybae* to *S. melanodermatum* and 61 mutational steps to *S. doceanum*.

Regarding the outgroup species, the analysis revealed significant genetic distances. Specifically, between *S. melanodermatum* and the outgroup species, *Brachyplatystoma rousseauxii* and *Zungaro zungaro*, 104 and 113 mutational steps were observed, respectively. These findings provide insights into the genetic divergence and relationships among the studied species, emphasizing notable genetic distances and relationships within and between species.

Phylogenetic Analysis

The topology resulting from the analyses using the BI (Bayesian Inference) and ML (Maximum Likelihood) methods showed significant similarities (Figure 4). The genus *Steindachneridion* was determined to be monophyletic for the four species examined in this study, grouped in a highly robust clade supported by a high posterior probability. *Steindachneridion scriptum* from the Tibagi and Uruguay Rivers exhibited consistent clustering into two distinct clades, supported by bootstrap and posterior probability values. *Steindachneridion doceanum* grouped with *S. parahybae*, while *S. melanodermatum* formed a single clade. The species *B. rousseauxii* and *Z. zungaro* clustered together, comprising the sister group to all the analyzed species.

----- Figure 4 here -----

Annotation and Characterization of Haplotypes of *S. melanodermatum*

Partial sequencing of the mitochondrial *D-loop* gene resulted in an array of 849 base pairs, situated between positions 15,651 and 16,500 within the mitogenome of *S. melanodermatum* (ON408064) (Figure 5). The 27 additional *D-loop* haplotypes (Haplo1 to Haplo27) retrieved from references

MZ672137-MZ672163 encompassed positions 15,590 to 16,460 within the mitogenome (Haplo1), constituting a sequence length of 870 base pairs (depicted in Figure 3).

----- Figure 5 here -----

Alignment of these obtained sequences with those available in Genbank revealed an overlap spanning 809 base pairs (from positions 15,651 to 16,460), flanked by two non-overlapping fragments situated upstream (positions 15,590 to 15,650) and downstream (positions 16,461 to 16,500) the overlapping region (as illustrated in Figure 4). Notably, these non-overlapping fragments represent non-conserved regions within the sequences and did not influence the identification of *S. melanodermatum* haplotypes.

This comprehensive alignment and analysis provide clarity on the specific regions used for sequence comparisons, underscoring the conserved nature of certain segments and their role in facilitating the accurate identification of *S. melanodermatum* haplotypes despite the presence of non-overlapping fragments.

DISCUSSION

Genetic Diversity Unveiled: A glimpse into *Stendachneridion* is enigmatic variability

The exploration of genetic diversity among the four *Steindachneridion* species has revealed a clustering mosaic of haplotypic variation, boasting substantial diversity levels (h=0.97-1.00 – Tab. 1). Notably, our methodologies, akin to studies on *S. scriptum* from the Uruguay River (*Paixão et al., 2018*) and *S. melanodermatum* from the Iguaçu River (*Souza-Shibatta et al., 2021*), resonate with the findings of these investigations. This revelation assumes particular intrigue given the status of these species as endemics (*Garavello, 2005*), believed to inhabit deep wells and facing looming extinction threats (*ICMBio, 2014*).

Our analyses, spanning haplotype networks and Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetics, delineated *Steindachneridion scriptum* populations from the Tibagi and Uruguay rivers into distinct Molecular Operational Taxonomic Units (MOTUs) (Figures 1 and 3). This corroborates prior findings in the Upper Uruguay River (*Paixão et al., 2018*). The presence of substantial genetic structure, coupled with evidence of departure from neutrality (as indicated by the R2 statistic and Fs statistic's results, respectively), underscores the complexity within these populations.

Remarkably, the discovery of numerous new haplotypes across the four Steindachneridion species signals an untouched reservoir of genetic variability within the genus. While previous studies, such as the one by (*Fonseca et al., 2017*), employing microsatellite markers, revealed low heterozygosity rates in *S. parahybae*, the conundrum of how intrapopulation genetic variability is maintained in *Steindachneridion* remains unresolved.

The emerging kinship dynamics among *Steindachneridion* species paint a clearer picture. Our haplotype network asserts a robust genetic affinity between *S. melanodermatum* and *S. scriptum* from the Uruguay River. These findings echo the conclusions drawn by (*Souza-Shibatta et al., 2021*), who, though employing different methodologies (six microsatellite markers in a larger

sample size (N=95)), reveled analogous connections between these species. Despite *S. melanodermatum*'s seemingly uncompromised genetic diversity, clues pointing to a recent bottleneck were evident in microsatellite data (*Matoso et al., 2011*). This study expands on previous knowledge, revealing 16 novel haplotypes for *S. melanodermatum*, illuminating the pressing need for deeper investigations into the species' genetic diversity.

Our discovery of several new haplotypes within the studied *Steindachneridion* species underscores the potential undervaluation of their genetic diversity. The cohesive groupings observed in the haplotype network, coupled with BI and ML analyses, accentuate each species' distinctiveness as a single Operational Taxonomic Unit (OTU), warranting due consideration for conservation efforts. Additionally, our findings, aligning with those by (*Fonseca et a., 2017*) in *S. parahybae*, highlight the conundrum posed by the combination of low nucleotide diversity and high haplotypic diversity, hinting at a demographic surge following a period of diminished effective population size.

Unraveling Evolutionary Enigmas: Insights into *Steindachneridion* is genetic tale

The exploration of morphological data and the genetic model data from nuclear and mitochondrial sources have unveiled a remarkable story within the Pimelodidae family (*Lundberg & Littmann, 2003*) constituting a monophyletic clade (*Sulivan et al., 2013*). Similar to this, monophyly is supported for the species of *Steindachneridion* (*Garavello, 2005*) positioning these fish as a basal lineage among pimelodids (*Lundberg et al., 2011*). Such evidence beckons for a deeper comparative and kinship investigation, particularly within the *Steindachneridion* genus. Leveraging Bayesian Inference (BI) and Maximum Likelihood (ML) analyses on 38 mitochondrial *D-loop* gene sequences from our studied species yielded robust clades, solidifying our results with a high-reliability index (Figure 3). This analytical exploration was empowered by the comprehensive mitochondrial genome of *S. melanodermatum* (*Silva et al., 2024*), pivotal for concatenating nucleotide sequences in our phylogenetic algorithms for BI and ML (Figure 4).

Our investigations, rooted in the topology of the Pimelodidae (*Lundberg & Akama, 2005*), showcased *Brachyplatystoma rousseauxii* and *Zungaro zungaro* as outgroups, delineating the phylogenetic relationships among examined *Steindachneridion* species, distinctly positioned outside these clusters. Internally, these clusters identified within the four *Steindachneridion* species under scrutiny stand as conclusive evidence, reverberating uniform biogeographic patterns. The geological dynamics of the Guiana and Brazilian continental shields, coupled with the drainage patterns along South America's east coast, have significantly shaped the river systems (*Ribeiro, 2006; Lundberg, 1998*). Notably, macrodome uplift, rifts, vertical movements, and erosive actions along the east bank have been pivotal in shaping the distribution of freshwater ichthyofauna in these regions. The observed biogeographic patterns (*Ribeiro, 2006*) underscore taxa with endemic coastal distributions maintaining phylogenetic ties with neighboring ichthyofauna, hinting at *Steindachneridion is* integration within this context. These fish, harboring endemic distributions across coastal basins (three of their six species) while spanning the Upper

Paraná basins, including the Iguaçu River and Uruguay River, exhibit kinship connections with *Zungaro*, *Sorubim*, *Pseudoplatystoma*, and the Amazon basin-dwelling *Brachyplatystoma*.

The observed clustering between *S. doceanum* + *S. parahybae* and *S. melanodermatum* + *S. scriptum* could be attributed, in part, to biogeographic features along Brazil's east coast, perhaps associated with the proximity of these species' river headwaters. Comparable findings in the 16S gene sequences of *Hoplias malabaricus* (*Dergman et al., 2002*) unveiled intertwined relationships across basins like Doce, Paraíba do Sul, and Grande, remnants of a shared past during the Plio-Pleistocene. The ichthyofauna of these basins is currently a reflection of a relict fauna that still bears similarities to one another (*Torres et al., 2008; Menezes et al., 2008*). Erosive retreats and headwater capture events might have sculpted the ichthyofauna relationships across these basins (*Ribeiro, 2006*).

Steindachneridion is included in the National List of Aquatic Vertebrates and Fish Threatened with Extinction (*ICMBio, 2018*) highlighting its conservation urgency. Species like *S. punctatum* and *S. amblyurum*, absent in our study due to sampling difficulties, emphasize their precarious status and the challenges in preserving them.

This study aims to illuminate the evolutionary dynamics within this fish group, even though numerous questions regarding the phylogenetic relationships of the *Steindachneridion* genus persist. Since the diversification of Siluriformes in the Neotropical region came before the division of South America from other continental blocks (*Pinna, 1998*). Thus, to achieve a more comprehensive phylogenetic and biogeographic scenario, comparative data with taxa beyond the Neotropical region are imperative. Future research on Sorubiminae's systematics promises to elucidate the phylogenetic intricacies of basal lineages within this group, potentially fortifying conclusions derived herein.

Four of the six known *Steindachneridion* species were central to our study. *S. punctatum*, unfortunately, is no longer found in the wild. Beyond contributing to the evolutionary narrative of this endangered endemic genus, our primary aim was to unveil the latent genetic variability within these species. Our crucial takeaway underscores distinct Molecular Operational Taxonomic Units (MOTUs) among *Steindachneridion* species, advocating for their conservation. Remarkably, recent events might have influenced genetic differentiation, hinting at intraspecific variability suggestive of speciation processes, demanding systematic consideration, as exemplified in the case of *S. scriptum*.

CONCLUSIONS

In conclusion, the study of genetic diversity within the *Steindachneridion* genus has revealed a wide range of variability, highlighted by distinct Molecular Operational Taxonomic Units (MOTUs) among species. This discovery of genetic intricacies not only provides insight into the evolutionary dynamics within this group of fish, but also emphasizes the urgent need for conservation efforts. As we navigate through the enigmatic evolutionary tale of *Steindachneridion*, further research remains imperative to fully comprehend the phylogenetic intricacies and biogeographic context of these basal lineages within the Pimelodidae family.

Furthermore, the identification of new haplotypes and detection of intraspecific variability suggest ongoing speciation processes. This highlights the need for systematic consideration and proactive conservation measures, particularly in light of the looming extinction threats faced by these endemic species.

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ADDITIONAL INFORMATION AND DECLARATION

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Competing Interests

The authors declare no conflict of interest.

Authors Contribution

Conceptualization, D.A.M and R.F.A.; Methodology, D.A.M.; H.C.M.S; Data Analysis, D.A.M; F.D.D.P.; A.L.F.J; F.P.F.; R.U. Investigation and data analysis, D.A.M; Writing – Original Draft Preparation, D.A.M.; Writing – Review & Editing, D.A.M, R.F.A, F.D.D.P.; Supervision, R.F.A.; Project Administration, R.F.A; Funding Acquisition, R.F.A. All authors have read and agreed to the published version of the manuscript.

Animal Etics

The animal study protocol was approved by the Ethics Committee for Animal Use Process CEUA: 0769342/2021

Data Aviability

DNA sequences used in this study are deposited in GenBank (accession numbers available in Material and Methods section).

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Figure 1

Geospatial analysis of *Steindachneridion* species distribution across South American river basins.

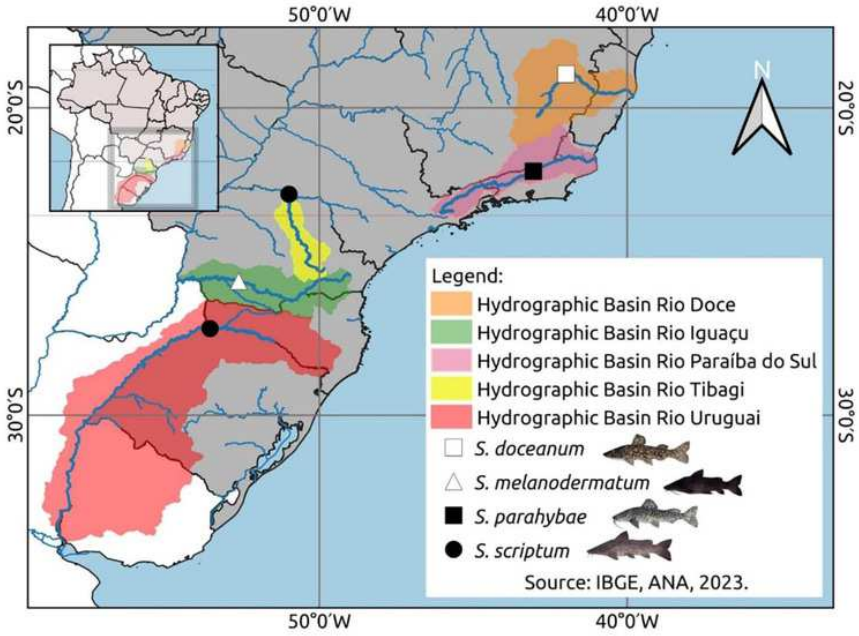

Figure 1. Geospatial analysis of *Steindachneridion* species distribution across South American river basins.

Figure 2

Haplotype network depicting mitochondrial D-loop gene variation among *Steindachneridion* species (Pimelididae) from South America.

Haplotypes are represented for each species and their respective river basins: *S. doceanum* from the Rio Doce basin (orange); *S. parahybae* from the Rio Paraiba do Sul basin (pink); *S. scriptum* from the Rio Tibagi and Rio Uruguay basins (yellow and red, respectively); *S. melanodermatum* from the Rio Iguaçu basin (green). The outgroup species are depicted in black: *Zungaro zungaro*; *Brachyplatystoma rousseauxii* (haplotype D31).

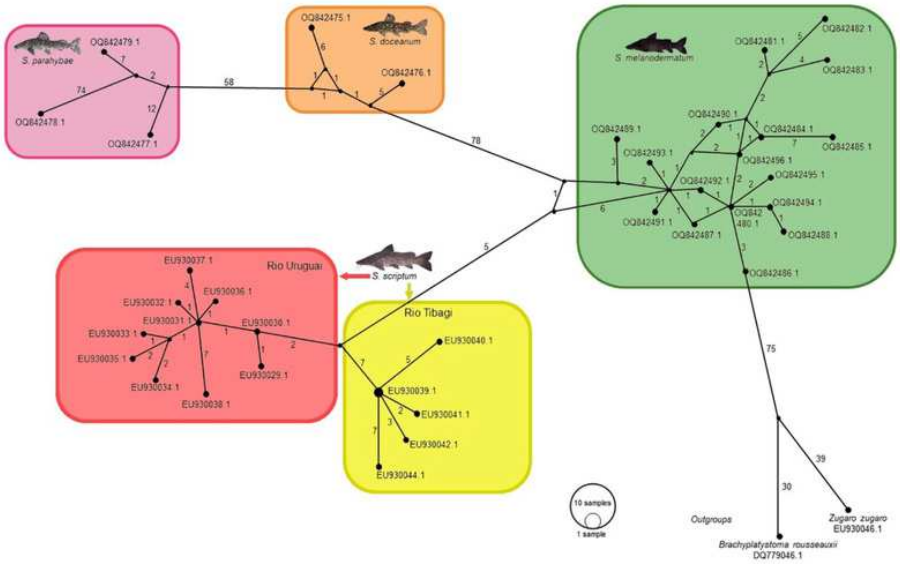

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Figure 3

Phylogenetic topology and genetic distances (HKY model) among *Steindachneridion* species sourced from South American rivers utilizing mitochondrial D-loop gene data.

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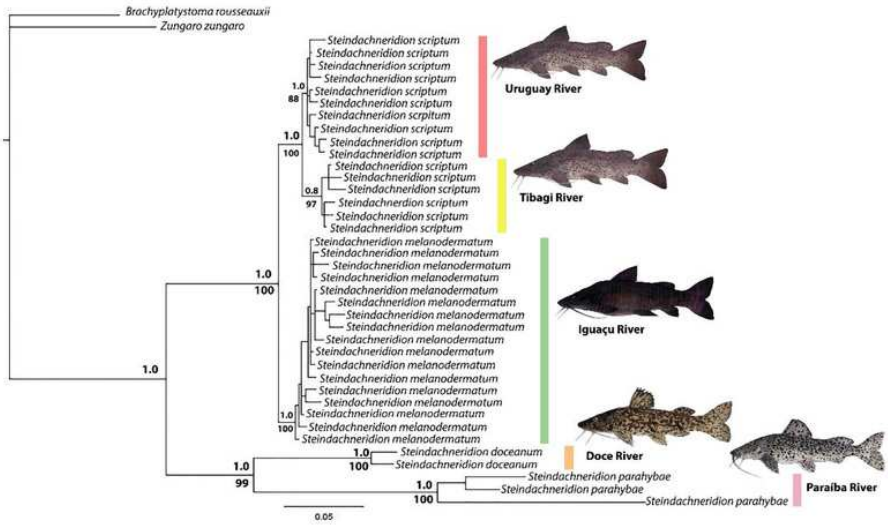

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Figure 4

Distribution of mitochondrial D-loop gene barcodes among *S. melanodermatum* samples sourced from South American rivers, alongside sequences available in GenBank mitogenomes (accession ON408264).

Base pair (bp) positions are indicated. Haplotypes are designated as Haplo1 to 27 - MZ672137 to MZ672163 (refer to Souza-Shibatta et al., 2021) and Haplo28 to 44 - OQ842475 - OQ842496 (from this study). GenBank accessions include Haplo1 - ON408064 (accepted by Silva et al., unpublished data).

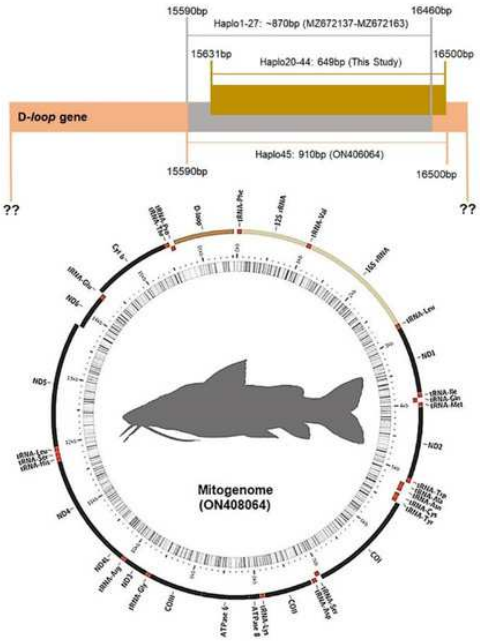

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Figure 5

Haplotype network illustrating the mitochondrial D-loop gene variation in *Steindachneridion melanodermatum* (S.m, n= 18) sourced from South American regions.

The identified haplotypes range from Haplo28 to Haplo44 (sequences OQ842475 - OQ842496, obtained in this study), alongside GenBank accessions such as Haplo1 - ON408064 (refer to Silva et al., unpublished data). Haplotypes not detected are represented by black circles.

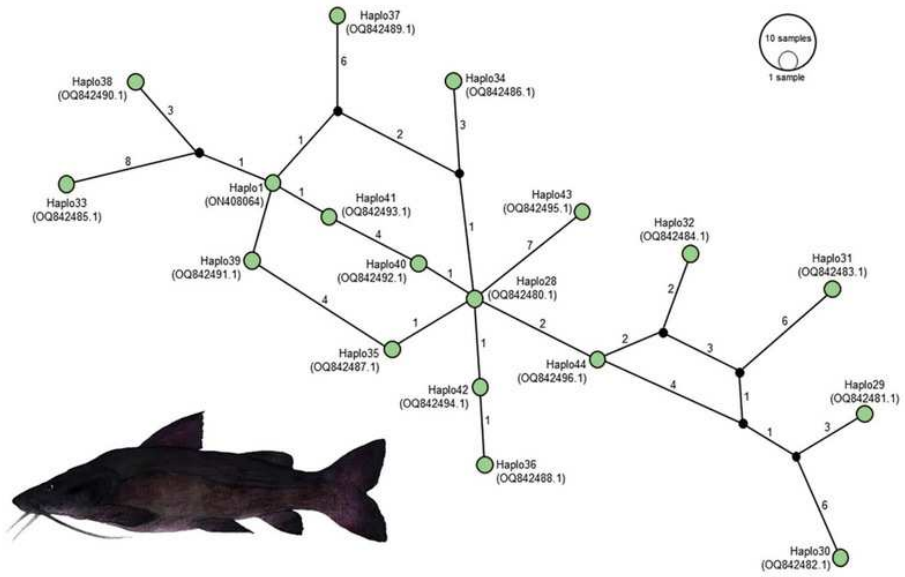

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Table 1(on next page)

Genetic diversity characterization for *Steindachneridion* fishes (Pimelodidae) in South America.

N – number of individuals examined; *n* – total number of sites; *S* = number of segregating sites; *Nh* = number of haplotypes; *h* - haplotype diversity; *π* = nucleotide diversity; *D* – Tajima's neutrality test; *Fs*—Fu's neutrality test; *R* - neutrality test [21]; *SD* – Standard Deviation; n/c – not calculated; ns – not significant ($p > 0.05$); * significant ($p < 0.05$),** highly significant ($p < 0.01$).

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Sampling	***N***	***n***	***S***	***Nh***	***h* ± *SD***	**π ± *SD***	***D***	***Fs***	**R_2**
Steindachneridion (Total-mean)	38	861	237	36	0,997 ±0,007	0,07254 ±0,01656	-1,69962 ns	0,444 ns	0,162 **
S. scriptum	16	849	58	15	0,992 ±0,025	0,01668 ±0,00217	-1,07389 ns	0,450 ns	0,162 **
Tibagi River	6	843	25	6	1,000 ±0,096	0,01205 ±0,00220	-0,54595 ns	0,451 ns	0,162 **
Uruguai River	10	849	29	9	0,978 ±0,054	0,00777 ±0,00158	-1,90383 *	0,439 ns	0,162 **
S. melanodermatum									
Iguaçu River	17	846	43	16	0,993 ±0,023	0,01093 ±0,00156	-1,33687 ns	0,437 ns	0,162 **
S. doceanum									
Doce River	2	814	24	2	1,000 ±0,500	0,03011 ±0,01506	n/c	0,363 ns	0,161 **
S. parahybae									
Paraíba do Sul River	3	658	117	3	1,000 ±0,272	0,13060 ±0,04295	n/c	0,348 ns	0,161 **