Neotropical Ichthyology

Original article

https://doi.org/10.1590/1982-0224-2023-0118

Cytogenetic and molecular studies in species of the Ancistrini tribe from Southern Brazil

Marcos Otávio Ribeiro[1], Isabelle Pereira Mari Ribeiro[2], Diego Mauro Carneiro Pereira[1], Thais Aparecida Dulz[1], Claudio Henrique Zawadzki[3,4], Rafael Bueno Noleto[1], Carla Andreia Lorscheider[1], Alessandra Valéria de Oliveira[2,4] and Ana Luiza de Brito Portela Castro[2,4]

Correspondence:
Marcos Otávio Ribeiro
otaviomarcos753@gmail.com

The southern region of Brazil is rich in hydric and biogeographic resources, contributing to the formation of distinct ichthyofaunistic niches and facilitating the isolation of some species. Despite the great ecological importance, there are few cytogenetic and molecular studies on the ichthyofauna of these basins. Therefore, specimens of *Ancistrus abilhoai* and *Hemiancistrus fuliginosus* were analyzed by combining cytogenetic and mitochondrial markers. Cytogenetic analysis revealed a diploid number of 2n = 48 for *A. abilhoai* and 2n = 56 for *H. fuliginosus* and Sites rDNA (by fluorescent *in situ* hybridization-FISH) were identified with 18S and 5S probes in synteny in pair 16 of *A. abilhoai*. At the same time in *H. fuliginosus*, these sites are located in separate pairs. Considering the *Ancistrus* cluster, based on COI molecular data, specimens of *A. abilhoai* were close to *A. cirrhosus* having as sister group *A. multispinis* and *A. brevipinnis*. Regarding *Hemiancistrus*, *H. fuliginosus* specimens showed the same haplotype as the sequences of this species, available in the database, forming a distinct clade with *H. aspidolepis* as a sister group. The results of our work helped to better define the taxonomic status of *A. abilhoai* and *H. fuliginosus*, species endemic to southern Brazil and which have few studies within their respective genera.

Keywords: *Ancistrus abilhoai*, COI, Cytotaxonomy, *Hemiancistrus fuliginosus*, Uruguay River.

Submitted December 21, 2022
Accepted January 26, 2024
by Marcos Mirande
Epub April 19, 2024

1 Centro de Exatas e Biológicas, Universidade Estadual do Paraná, Praça Coronel Amazonas s/n, Centro, 84600- 185, União da Vitória, PR, Brazil. (MOR) otaviomarcos753@gmail.com (corresponding author), (RBN) rafael.noleto@unespar.edu.br, (DMCP) diegom8135@gmail.com, (TAD) thais.dulz@ies.unespar.edu.br, (CAL) carla.lorscheider@unespar.edu.br.

2 Departamento de Biotecnologia, Genética e Biologia Celular, Universidade Estadual de Maringá, Av. Colombo, 5790, Jardim Universitário, 87020-900 Maringá, PR, Brazil. (AVO) avoliveira@uem.br, (ALBPC) albpcastro@nupelia.uem.br, (IPMR) isa_mari93@hotmail.com.

3 Departmento de Biologia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900 Maringá, PR, Brazil. (CHZ) chzawadzki@hotmail.com.

4 Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicutura (Nupelia), Av. Colombo 5790, Jardim Universitário, 87020-900 Maringá, PR, Brazil.

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 22, no. 1, Maringá 2024

Official Journal of the Sociedade Brasileira de Ictiologia

A região sul do Brasil é rica em recursos hídricos e biogeográficos, contribuindo para a formação de nichos ictiofaunísticos distintos facilitando o isolamento de algumas espécies. Apesar da grande importância ecológica, existem poucos estudos citogenéticos e moleculares sobre a ictiofauna dessas bacias. Por isso, espécimes de *Ancistrus abilhoai* e *Hemiancistrus fuliginosus* foram analisados através da combinação de marcadores citogenéticos e mitocondriais. A análise citogenética revelou um número diploide de 2n = 48 para *A. abilhoai* e 2n = 56 para *H. fuliginosus* e foram identificados sítios de DNAr (por hibridização *in situ* fluorescente-FISH) com sondas 18S e 5S, em sintonia no par 16 de *A. abilhoai*, enquanto em *H. fuliginosus* estes sítios estão localizados em pares separados. Considerando o *cluster Ancistrus*, com base nos dados moleculares COI, os espécimes de *A. abilhoai* ficaram próximos de *A. cirrhosus*, tendo como grupo irmão *A. multispinis* e *A. brevipinnis*. Em relação a *Hemiancistrus*, os exemplares de *H. fuliginosus* apresentaram o mesmo haplótipo das sequências desta espécie, disponíveis no banco de dados, formando um clado distinto com *H. aspidolepis* como grupo irmão. Os resultados do nosso trabalho auxiliaram na melhor definição do status taxonômico de *A. abilhoai* e *H. fuliginosus*, espécies endêmicas do sul do Brasil e que exibem poucos estudos dentro de seus repctivos gêneros.

Palavras-chave: *Ancistrus abilhoai*, *COI*, Citotaxonomia, *Hemiancistrus fuliginosus*, Rio Uruguai.

INTRODUCTION

The diversity of fish species in the Neotropical region is considered one of the greatest in the world (Vari, Malabarba, 1998). Montoya Burgos (2003) in his study of fishes of this region correlated the historical biogeographic aspects and the implications in the diversification of Neotropical species. Cladogenic hydrogeological events, that occurred milions of years ago were fundamental in the diversification of species, dividing and displacing river courses, associated with repeated incursions and regressions of the sea level under the continent, producing numerous vicariant events, which culminated in biotic enrichment (Lundberg, 1998; Montoya Burgos, 2003).

Brazil, which is part of the Neotropical region, is divided into twelve hydrographic regions according to the SIRHESC (2021). The basin of the Iguaçu River and the Uruguay River includes portions of the states of Paraná, Santa Catarina, and Rio Grande do Sul, showing great importance in water resources. The peculiar conditions of these basins propitiate the formation of endemic species restricted to small areas, streams, or micro-basins. Among the various fish families in this region, the Loricariidae is the most representative family within Siluriformes and currently comprises 1,048 valid species (Fricke *et al*., 2023). Taxonomic problems within the Loricariidae are recurrent with new species. Among Loricariidae, the Ancistrini clade stands out, composed of numerous genera with high morphological diversity constantly undergoing systematic reformulations (Lujan *et al*., 2015).

Armbruster *et al*. (2015) described three species of the genus *Peckoltia* Miranda Ribeiro, 1912 and proposed a taxonomic revision for *Hemiancistrus* Bleeker, 1862 and related genera based on molecular phylogeny analyses. According to the authors, molecular phylogeny suggested that the only species that should be kept in *Hemiancistrus* is *Hemiancistrus medians* (Kner, 1854) (type-species), and the other members of the taxa that do not have well-established genera will be recognized as species groups in '*Hemiancistrus*' until they can be further examined. In addition, Armbruster *et al*. (2015) identifed three species groups for *Hemiancistrus*, such as *H. chlorostictus* Cardoso & Malabarba, 1999, *H. guahiborum* Werneke, Armbruster, Lujan & Taphorn, 2005, and *H. landoni* Eigenmann, 1916. Chromosome studies in the genera *Ancistrus* Kner, 1854 and *Hemiancistrus* also reflect the taxonomic complexity of these groups, especially in *Ancistrus*, considered the most diverse among the Ancistrini. In *Ancistrus*, a variable chromosome range is detected from 2n = 34 in *Ancistrus cuiabae* Knaack, 1999, (Mariotto *et al*., 2009) to 2n = 54 in *Ancistrus claro* Knaack, 1999, (Marioto *et al*., 2013). *Ancistrus* exhibits peculiar chromosomal dynamics presenting diverse sex-determination systems such as ZZ/ZW in *A. ranunculus* Muller, Rapp Py-Daniel & Zuanon, 1994, (de Oliveira *et al*., 2007), *A. taunayi* Miranda Ribeiro, 1918 (Konerat *et al*., 2015), XX/XY system in *A.* cf. *dubius* (Mariotto, Miyazawa, 2006) and in two *Ancistrus* populations from the Paraná River basin, PR (Prizon *et al*, 2017), and multiple systems such as XX/XY1Y2 in *Ancistrus* sp. Balbina (de Oliveira *et al*., 2008), XX/X0 in *Ancistrus* n. sp. 1 (Alves *et al*., 2006) and Z1Z1Z2Z2Z2/Z1Z2W1W2 in *Ancistrus* sp. Barcelos (de Oliveira *et al*., 2008). *Hemiancistrus*, currently exhibits few cytogenetic descriptions with only five records: *H. spilomma* Cardoso & Lucinda, 2003, *H. spinosissimus* Cardoso & Lucinda, 2003 (de Oliveira *et al*., 2006), *Hemiancistrus* sp. (Artoni, Bertollo, 2001) and *H. punctulatus* Cardoso & Malabarba, 1999, (Rubert, 2011) being all species with 2n = 52 chromosomes and predominance of chromosomes of metacentric and submetacentric types. In addition, ZZ/ZW sex determination system was recorded in *H. spilomma* (de Oliveira *et al*., 2006).

Gugloski *et al*. (2020) in a review of cytogenetic data listed 53 species of *Ancistrus* revealing its great karyotypic diversity in diploid number, formula, and other chromosomal markers, including many species with taxonomic status not yet well defined. These data demonstrate the need to expand the analyses, not only on cytogenetics, but integrated with taxonomic revisions and DNA molecular analyses for more accurate identification of species of this group. Prizon *et al*. (2017) differentiated five *Ancistrus* lineages from the Paraná River basin using DNA barcode and cytogenetic data thus contributing to the record of an underestimated diversity in this genus for the upper Paraná River basin (Paraná State). *Ancistrus agostinhoi* Bifi, Pavanelli & Zawadzki, 2009, *A. mullerae*, and *A. abilhoai* were described by Bifi *et al*. (2009), occurring in the lower and middle Iguaçu River respectively, between the States of Paraná and Santa Catarina. Subsequently, *A. abilhoai*, was described cytogenetically by Ribeiro *et al*. (2015), this being considered endemic by Baumgartner *et al*. (2012). Therefore, considering the cytotaxonomic complexity of species of *Ancistrus* and *Hemiancistrus* genera, in this study, we present cytogenetic and molecular data for two populations of *A. abilhoai* and one population of *H. fuliginosus* Cardoso & Malabarba, 1999, collected in rivers of the Iguaçu river basin, whose results compared to other species of the respective genera, will constitute important references in cytotaxonomic, karyoevolutionary aspects and supports molecular phylogeny in these groups.

MATERIAL AND METHODS

Biological samples. Specimens from the genera *Ancistrus* and *Hemiancistrus* (Tribe Ancistrini, Loricariidae) were used in cytogenetic and molecular studies (Fig.1). For the genus *Ancistrus*, specimens from two populations were collected: *A. abilhoai* from Iratim River, municipality of General Carneiro, PR, Iguaçu River basin (26°19'44.21"S 51°34'39.37"W), totaling 13 males and six females and one population of *A. abilhoai* from river of Pardos, district of Santa Cruz do Timbó, Porto União, SC, Iguaçu River basin (26°26'39.08"S 50°58'44.98"W), totaling four females and one male. *Hemiancistrus fuliginosus* was collected in Fragosos River, municipality of Concórdia, SC, upper Uruguay River basin (27°13'27.7"S 52°10'07.9"W), totaling five females and four males (Fig. 1). After collection, the specimens were transported in aerated boxes to the fish cytogenetics laboratory of the Universidade Estadual do Paraná (UNESPAR), União da Vitória, PR. Some specimens were deposited in the ichthyological collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUPELIA), of the Universidade Estadual de Maringá, Paraná, Brazil: *H. fuliginosus* (NUP 21922), *Ancistrus abilhoai* General Carneiro, PR (NUP 23486) and *A. abilhoai* Santa Cruz do Timbó, SC (NUP 23551). The samples were anesthetized and euthanized by overdosing with clove oil (Griffiths, 2000).

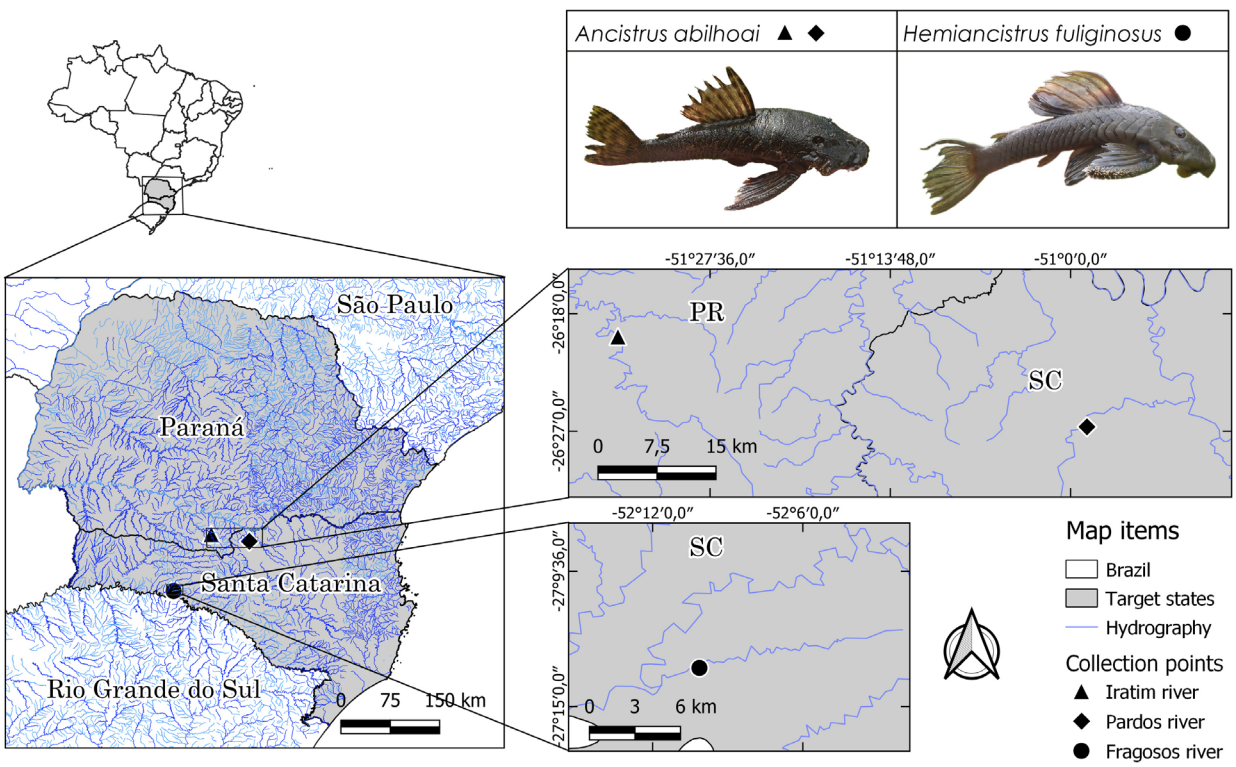

FIGURE 1 | Geographic location of the points in southern Brazil where the specimens were collected. Triangle corresponds to the populations of *Ancistrus abilhoai* Iratim River, PR and the rectangle to the population of *A. abilhoai* Pardos River, SC. Losango symbolizes the *Hemiancistrus fuliginosus* population collected in Fragosos River, SC. Blue lines represent the microbasins and drainage areas.

Cytogenetic procedures. Mitotic chromosomes were obtained from kidney cells according to the methodology proposed by Bertollo *et al*. (1978). The silver nitrate impregnation technique revealed nucleolus organizing regions (NORs) (Howell, Black, 1980). Constitutive heterochromatin regions were determined by the C-banding technique (Sumner, 1972), and stained with propidium iodide (Lui *et al*., 2012).

Physical mapping of 5S and 18S rDNA sequences was performed by fluorescence *in situ* hybridization (FISH) technique according to Pinkel *et al*. (1986), using probes obtained from *Megaleporinus obtusidens* (Valenciennes, 1837) (Martins, Galetti, 1999) and *Prochilodus argenteus* Spix & Agassiz, 1829, (Hatanaka, Galetti, 2004). Probes were marked by Nick Translation with biotin-16-dUTP (rDNA 5S) and digoxigenin-11-dUTP (rDNA 18S). The hybridization process was conducted under high-stringency conditions (77%). Signals were detected using anti-digoxigenin-rhodamine, conjugated to 18S rDNA probes, and avidin-FITC conjugated to 5S rDNA probes. The chromosomes were counterstained with DAPI. Image capture was realized using a fluorescence microscope model Zeiss Axio Lab A1. For the elaboration of karyotypes, chromosomes were paired in groups of (m) metacentric, (sm) submetacentric, (st) subtelocentric and (a) acrocentric according to Levan *et al*. (1964). The fundamental number (FN) was calculated according to the chromosome arm number, metacentric, submetacentric and subtelocentric chromosomes were considered as containing two arms and acrocentric as one arm.

DNA extraction, amplification, and sequencing. Total genomic DNA extraction was performed from liver samples using the Promega Wizard ®Genomics kit, following the manufacturer's instructions. After extraction, DNA was quantified using 1% agarose gel electrophoresis, by comparison with lambda DNA of known concentration. The mitochondrial region of cytochrome *c* oxidase I (COI), was partially amplified using the primers L6448-F2 (5'-TCGACTAATCATAAAGATCGGCGCAC-3') and H7152 (5'-CACCTCAGGGGTGTCCGAARAAYCARA-3') described by Ivanova *et al*. (2007).

The polymerase chain reaction (PCR) consisted of Tris-KCl [20 mM Tris-HCl (pH 8.4), 50 mM KCl], $MgCl_2$ (1.5 mM), primers (2.5 μM each), dNTPs (0.1 mM each), DNA Taq Polymerase (1U) and template DNA at a concentration of 10ng/ul to make up a final volume of 25 μl. Conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for the 30s, 52°C for 30s, and 72°C for 1 min with a final elongation cycle at 72°C for 10 min. Amplicons were checked on 1% agarose gel by electrophoresis and purified with polyethylene glycol (Rosenthal *et al*., 1993). For the sequencing reaction, the Big Dye Terminator kit was used. The sequencing reactions and sequencing were performed at private company, using the ABI-3500 automated sequencer.

Molecular Analysis. The sequences obtained were edited and aligned by Clustal W using BioEdit (Hall, 1999) and MEGA 7.0 (Kumar *et al*., 2016) software, respectively. In addition to the sequences obtained in this work, sequences available in Genbank for *Ancistrus* and *Hemiancistrus* species (except *Ancistrus* sp. and *Hemiancistrus* sp.) were used for haplotype selection, performed by DnaSP 6 software (Rozas *et al*., 2017) (Tab. **S1**). The analysis did not use sequences with reduced size, compromising the

final aligment. Genetic distances values were calculated between groups of species and between haplotypes by the Kimura-2-parameter model. Gene tree was constructed by the maximum likelihood method, with 1000 bootstrap resamplings, using MEGA 7.0 software. *Rhinelepis aspera* Spix & Agassiz, 1829 was used as an outgroup (MZ052007.1).

RESULTS

Karyotypic description. Specimens of *Ancistrus abilhoai* from Iratim River (General Carneiro, PR) and from Pardos River (Santa Cruz do Timbó, SC) showed 2n = 48 chromosomes in both sexes, with a karyotypic formula composed of 18m+8sm+12st+10a and fundamental number 86 (Fig. 2A). C-banding revealed few heterochromatic blocks being prominent in the centromeric region of most metacentric and submetacentric chromosomes, with strongly stained blocks standing out in the short arm extension of the submetacentric pair 16, this positive for the Ag-NORs pair (Fig. 2B). The 18S and 5S rDNA probes hybridized at the pair 16 coincident with Ag-NORs sites in both populations of *A. abilhoai*. In addition to the synteny observed between 18S and 5S sites an additional 5S rDNA site was evidenced in chromosomal pair 19, subtelocentric (Fig. 2C).

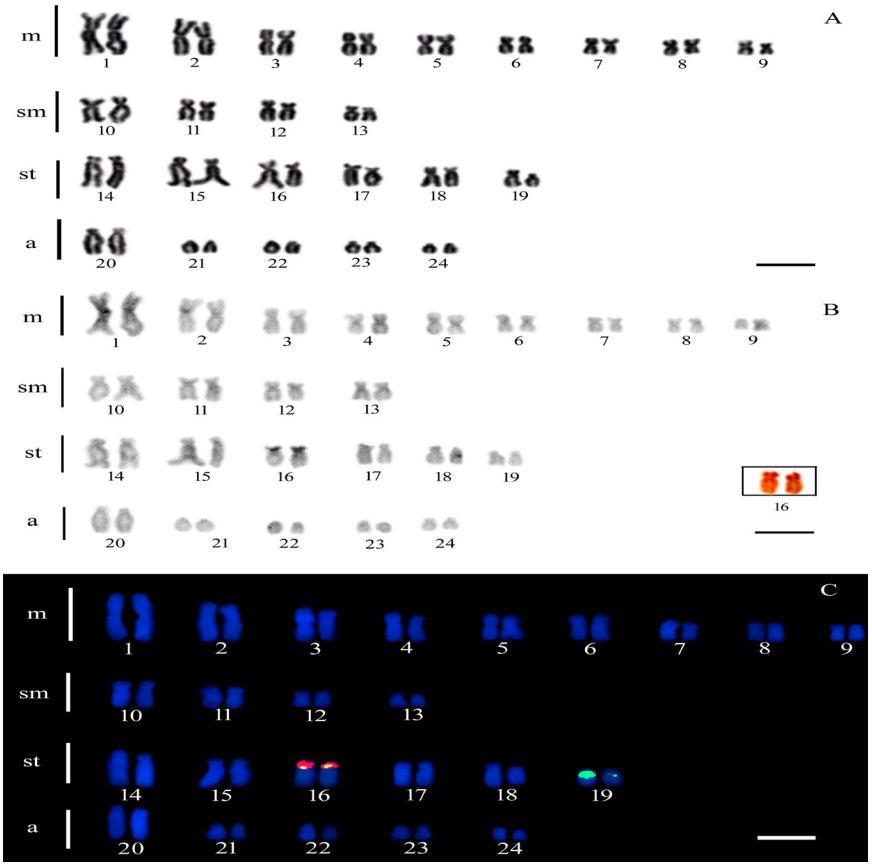

FIGURE 2 | Representative karyotype of both *Ancistrus abilhoai* populations. (**A**) Giemsa staining; (**B**) after C-banding, in box pair 16 carrying the Ag-NOR; (**C**) karyotype after double FISH with 18S (pink) and 5S (green) DNAr probes. Note the synteny of rDNA sites in pair 16 and an additional 5S rDNA site in pair 19. Scale bars = 10µm.

Hemiancistrus fuliginosus exhibited a diploid number of 2n = 56 chromosomes, with a karyotypic formula 22m+18sm+16st in males and females and a fundamental number of 112 (Fig. 3A). Ag-NOR sites were detected on the short arm of pair 12, in the proximal position (Fig. 3B, in box), coincident with heterochromatic blocks and 18S rDNA regions detected by FISH (Fig. 3C, pink signal). The 5S rDNA probe (green signal) hybridized to the pericentromeric region of pair 7 (Fig. 3C), coincident with heterochromatic blocks.

Molecular analysis. A total of 76 sequences of the COI gene, with 554 bp, after alignment and editing, were obtained: two for *Ancistrus abilhoai*, two for *Hemiancistrus fuliginosus* from the present study and 72 sequences available from GenBank. Due to the high number of sequences, the Kimura-2-parameter (K2P) distance was calculated between species groups (Tab. 1) and between haplotypes (Tab. **S2**). The different specimens of *Ancistrus abilhoai* (sampled in Iratim-General Carneiro, PR and Pardos-Santa Cruz do Timbó, SC), presented the same haplotype. In contrast, the specimens of *Hemiancistrus fuliginosus* showed 100% similarity to sequences of *H. fuliginosus* from the Genbank. No COI gene sequence for *A. abilhoai* was found in the database, this being the first deposit for the species.

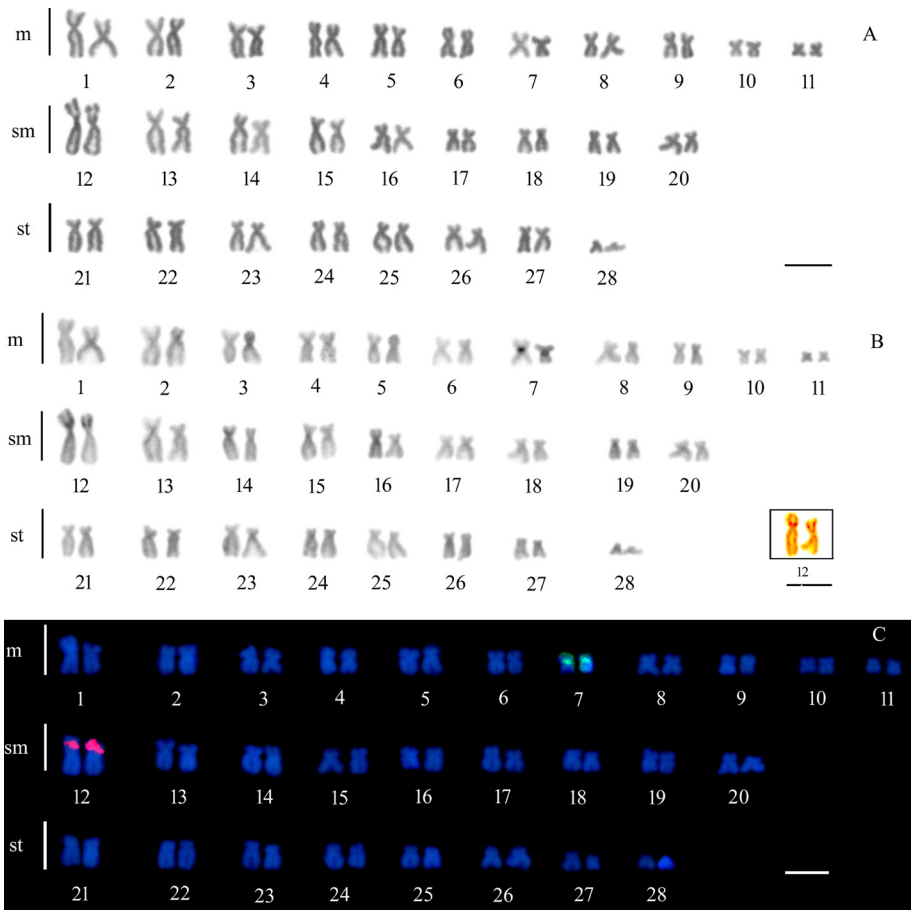

FIGURE 3 | Representative sequential karyotype of *Hemiancistrus fuliginosus*. (**A**) Giemsa staining; (**B**) after C-banding with Ag-NOR pair 12 in the box; (**C**) karyotype after FISH with 18S (pink) and 5S (green) rDNA probes in **C** note the C-positive and FISH markings on pairs 12 and 7. Scale bars = 10µm.

The mean K2P distances between *Ancistrus abilhoai* and the other *Ancistrus* species available in the database, ranged from 1.1% (with *A. cirrhosus* (Valenciennes, 1836)) to 9.6% (with *A.* cf. *leucostictus*). Among the *Hemiancistrus* species, *H. fuliginosus* was genetically closer to *H. aspidolepis* (Günther, 1867) (Tab. 1).

The COI genetic tree was built using the Hasegawa-Kishino-Yano model, representing the haplotypes obtained from the DNAsp program. The sequences obtained in the present study are marked with a triangle (Fig. 4). Two large clusters were formed, one constituted by specimens of *Ancistrus* and the other by *Hemiancistrus*. *Ancistrus abilhoai* grouped with *A. cirrhosus*, demonstrating the proximity of the two species and have as sister groups *A. multispinis* and *A. brevipinnis* (Regan, 1904). Regarding *Hemiancistrus*, *H. fuliginosus* from the present work present 100% similarity with others sequences of the same species available in the database, forming a distinct clade with *H. aspidolepis* as its sister group, and being more distant from *H. subviridis* Werneke, Sabaj Pérez, Lujan & Armbruster, 2005 and *H. medians*.

TABLE 1 | K2P interespecific genetic distances of the partial fragment of COI gene of *Ancistrus* and *Hemiancistrus* from de GenBank and the presente study. *Indicates the species described in this study.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *A. abilhoai**														
2. *A. aguaboensis*	0.069													
3. *A. brevipinnis*	0.038	0.083												
4. *A.* cf. *leucostictus*	0.096	0.094	0.083											
5. *A. chagresi*	0.094	0.114	0.104	0.112										
6. *A. cirrhosus*	0.011	0.072	0.042	0.095	0.100									
7. *A. cryptophthalmus*	0.065	0.058	0.073	0.093	0.102	0.068								
8. *A. dolichopterus*	0.079	0.100	0.090	0.083	0.103	0.084	0.090							
9. *A. multispinis*	0.034	0.076	0.029	0.095	0.101	0.035	0.066	0.088						
10. *A. spinosus*	0.090	0.115	0.097	0.117	0.032	0.097	0.094	0.100	0.105					
11. *A. temminckii*	0.072	0.081	0.064	0.024	0.094	0.075	0.072	0.066	0.076	0.101				
12. *H. aspidolepis*	0.168	0.164	0.167	0.149	0.184	0.166	0.160	0.145	0.168	0.179	0.148			
13. *H. fuliginosus**	0.150	0.168	0.152	0.154	0.185	0.150	0.161	0.149	0.155	0.178	0.148	0.060		
14. *H. medians*	0.176	0.179	0.180	0.152	0.189	0.174	0.167	0.151	0.176	0.179	0.156	0.119	0.109	
15. *H. subviridis*	0.166	0.159	0.166	0.161	0.168	0.161	0.160	0.160	0.156	0.169	0.154	0.131	0.116	0.127

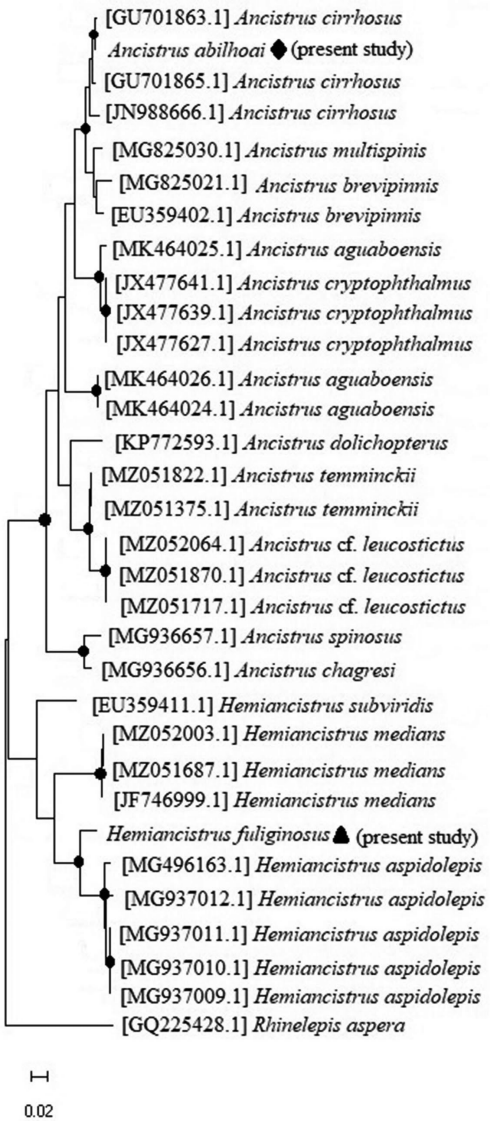

FIGURE 4 | Gene tree constructed by the maximum likelihood method from partial sequences of the COI gene of *Ancistrus* and *Hemiancistrus* species from GenBank and the present study. Black dots on branches represent support values above 85%. *Rhinelipis aspera* was used as an outgroup.

DISCUSSION

Cytogenetic analysis. Anicistrini is a tribe that shows great chromosomal diversity with a karyotypic range from 2n = 34 to 54 chromosomes (Bueno *et al*., 2018), and much of this diversity is evidenced in the genus *Ancistrus*. The 2n = 48 value detected in this study for *Ancistrus abilhoai* was found in a few species of this genus, as recorded in *A. ranunculus* (de Oliveira *et al*., 2007, Favarato *et al*., 2016) and the *A. abilhoai* population from the Iguaçu River (Ribeiro *et al*., 2015). Although the populations of *A. abilhoai* have the same diploid number (2n = 48), however the karyotypic formulas are distinct, being 18m+8sm+12st+10a for the populations of the present study and 22m+14sm+6st+6a for the Iguaçu River population (Ribeiro *et al*., 2015). These interpopulation structural variations suggest the occurrence of chromosome rearrangements, mainly pericentric

inversions that, with the centromere repositioning, change the chromosome morphology without changing the diploid number. Variations in inter and intraspecific karyotypic formulas also indicate the currency of structural rearrangements, such as translocations and pericentric inversions contributing to chromosome diversification in this group (Prizon *et al*., 2017). The notorious variation observed among the karyotypes of this group of fish possibly suggests that with biological and ethological aspects, given their preference for microhabitats, where they remain hidden in crevices or trunks, establishing territories and thus exhibiting low vagility, what could contribute to fixation of chromosomal rearrangements (de Oliveira *et al*., 2006).

The karyotype of *Hemiancistrus fuliginosus* is divergent from other descriptions for the genus. The diploid number of 2n = 52 chromosomes is a predominant value for many Ancistrini species (Bueno *et al*., 2018) and, therefore, a value of 2n = 56 chromosomes found in *H. fuliginosus* in this study exceeds the maximum value recorded for Ancistrini species of 2n = 54 for *Ancistrus claro*, *Ancistrus* sp. 1, and *Ancistrus* sp. 3, beyond how Glugoski *et al*. (2020) wrote them, the karyotype of *H. fuliginosus* exhibited a predominance of meta and submetacentric chromosomes, together with the other species described for this genus.

Considering the taxonomic complexity of Ancistrini genera, such as *Hemiancistrus*, cytogenetic studies have much to contribute to this group. According to the groups for *Hemiancistrus* proposed by Armbruster *et al*. (2015), *H. fuliginosus* belongs to the *H. chlorostictus* group. However, karyotypic data were presented only for *H. punctulatus*, also included in this group, which proves to be divergent in chromosome number and karyotypic formula from *H. fuliginosus*. Therefore, more *Hemiancistrus* species need to be analyzed, including the type species *H. medians*, for a better definition of karyotypic interrelationships in this group. Other descriptions exist for *Hemiancistrus* in southern Brazil: *H. votouro* Cardoso & da Silva, 2004, *H. meizospilos* Cardoso & da Silva, 2004, and *H. chlorostictus* Cardoso & Malabarba, 1999, but these descriptions are restricted only to morphological features, with an absence of cytogenetic data (Cardoso, Malabarba, 1999; Cardoso, da Silva, 2004).

In some *Ancistrus* species the location of chromosome-specific heterochromatic blocks can be helpful and collaborate in recognition of fusion points (Rosa *et al*., 2012; Barros *et al*., 2017; Glugoski *et al*., 2018) or in recognition of heteromorphic sex chromosomes (de Oliveira *et al*., 2007, 2008, 2009; Mariotto *et al*., 2011; Konerat *et al*., 2015; Favarato *et al*., 2016; Prizon *et al*., 2018; Schemberger *et al*., 2019). The presence of large heterochromatic blocks is a feature widely shared among *Ancistrus* species (Mariotto *et al*., 2011; Konerat *et al*., 2015; Favarato *et al*., 2016) also contributing to differentiation among their populations (Prizon *et al*., 2017, 2018), whereas the absence of conspicuous heterochromatic bands is described as an ancestral feature in Loricariidae (Ziemniczak *et al*., 2012). In *A. abilhoai*, its karyotype does not evidence heteromorphism of sex chromosomes, and only a single heterochromatic block on pair 16 stands out, co-localized with the 18S and 5S rDNA locus. Similarly, the karyotype of *H. fuliginosus* showed few heterochromatic blocks, except those co-localized with the 5S rDNA (pair 7) and 18S rDNA (pair 12). Indeed, for *Ancistrus*, co-localization of repetitive sequences (heterochromatin/ribosomal sites) indicates a strong correlation of these chromosomal domains with fragile sites in the genome, particularly involving the 5S rDNA sequences (Rosa *et al*., 2012; Barros *et al*., 2017; Glugoski *et al*., 2018; Glugoski *et al*., 2020), explaining part of the Robertsonian fusions in *Ancistrus* and *Hemiancistrus*.

The results of *in situ* hybridization with 18S and 5S rDNA in *A. abilhoai* were similar to those found in the *A. abilhoai* population from the Iguaçu River by Ribeiro *et al*. (2015), with the occurrence of synteny of 18S/5S rDNA. Glugoski *et al*. (2020), found synteny in *A. aguaboensis* Fisch-Muller, Mazzoni & Weber, 2001, Tocantins basin. Mariotto *et al*. (2011), analyzing *Ancistrus* from the Amazon, Paraguay, and Araguaia river basins, found synteny in: *A. claro*, *Ancistrus* sp. 08, *A.* cf. *dubius*, and *A.* sp. 06 Prizon *et al*. (2017) also found synteny in three of the five *A. cirrhosus* populations studied from the upper Paraná basin.

The syntenic condition of rDNA is widely observed in karyotypes of the family Loricariidae (Kavalco *et al*., 2004; Mariotto *et al*., 2011; Ziemniczak *et al*., 2012; Traldi *et al*., 2013; Bueno *et al*., 2014; Favarato *et al*., 2016; Barros *et al*., 2017). Synteny in the tribe Ancistrini is not exclusive to *Ancistrus*; Silva *et al*. (2021) pointed out synteny in *Peckoltia* sp. 3 Jarumã and Favarato *et al*. (2017) citogenetically described several *Ancistrus* species and found unique 5S rDNA sites in *A. dubius* Eigenmann & Eigenmann, 1889, *A. maximus*, Artoni, Zuanon, Zawadzki & Rapp Py-Daniel, 2015, *A. ranunculus* Muller, Rapp Py-Daniel & Zuanon, 1994, and multiples in *Ancistrus* sp. "Purus", *Ancistrus* sp. "Catalan" *A. dolichopterus* Kner, 1854, and *A.* aff. *dolichopterus*.

The involvement of 5S rDNA sequences in chromosomal diversification in *Ancistrus* has been proposed from observations of a highly dynamic distribution pattern, ranging from 1 to 13 pairs carrying these sequences (Glugoski *et al*., 2020). Disjunction of ribosomal sites caused by rearrangements and/or mobile genetic elements appears to be a common condition, among Neotropical fish species. The location of 5S rDNA sites in the proximal region of st/a chromosomes has been recognized as chromosome fusion sites, which in some species, may have an association with interstitial telomeric (ITS) sequences and heterochromatin (Rosa *et al*., 2012; Primo *et al*., 2017; Glugoski *et al*., 2018). Dispersion of 5S rDNA sites across the genome may result from copy duplications of this region and/or may be associated with retrotransposable elements. Prizon *et al*. (2018) evidenced the associations between Rex-3 elements to 18S and 5S rDNA sites, in different *Ancistrus* populations, from the upper Paraná River basin. For Mariotto *et al*. (2011) in *Ancistrus*, 5S rDNA variation has been attributed to genetic mechanisms such as pericentric inversions and unequal permutations. This results in the current diversity of marked pair numbers and chromosome types with 5S rDNA. Medeiros *et al*. (2016) pointed out that ribosomal site variation and wide distribution may characterize a derived state in this genus.

Molecular analysis. Sequences of the cytochrome *c* oxidase, subunit I (COI) gene have been frequently used in fish species identification, as well as in population studies contributing to taxonomic elucidations in complex groups (Hebert *et al*., 2003; Waugh *et al*., 2007). The results obtained in our work indicate that within the genus *Ancistrus*, *A. abilhoai* is genetically close to *A. cirrhosis*, presenting distance genetic values of 1.1%. Prizon *et al*. (2017), using this mitochondrial marker (COI) and associated with cytogenetic tools, pointed out five distinct lineages of *Ancistrus* of the upper Paraná River basin exhibiting genetic distances between 3 and 5%. These authors included a population from Arroyo San Juan (Misiones, Argentina), considered to represent the nominal *A. cirrhosus*, a single representative of the genus for the upper Paraná River basin (Langeani *et al*., 2007). Although, our results for *A. abilhoai* showed a genetic distance

of 1.1% with *A. cirrhosus*, the cytogenetic data of *A. cirrhosus* revealed divergence in a diploid number of 2n = 50 (Prizon *et al*., 2017) and in its karyotypic formula compared to *A. abilhoai* (2n = 48). These divergences corroborate a diversity in populations of *Ancistrus,* not yet fully resolved from a taxonomic point of view for this genus.

In Fig. 4 it is also observed that the specimens identified as *A. aguaboensis* grouped into two distinct clusters, one of them composed of two haplotypes of *A. aguaboensis* and the other with one haplotype of *A. aguaboensis* and three of *A. cryptophthalmus* Reis, 1987. The haplotypes described as *A. aguaboensis* shows a genetic distance of 7.94% from each other, while the distance for the *A. cryptophthalmus* haplotype is 1.74%. Findings like these demonstrate that within *Ancistrus* taxonomy-related problems are present, and the use of multiple tools in species identification is of fundamental importance for a more assertive description. Borba *et al*. (2019) using COI in *Ancistrus*, discriminated 7 lineages from the Amazon basin and 8 from the Paraguay basin, with an average distance of 8.4% between lineages, and two of these lineages, exhibited the same diploid number, of 2n = 54 chromosomes and with very similar morphology, however, the COI result, pointed distance of 3.3% between them.

The molecular and cytogenetic results of the present study helped in the identification and genetic characterization of the *Ancistrus* and *Hemiancistrus* species analyzed. The results for *A. abilhoai*, corroborated the pre-existing cytogenetic data for this species as analyzed by Ribeiro *et al.* (2015) and suggest that it is the same species, consisting in the main reference for *A. abilhoai*. However, the findings in our work, for populations of *A. abilhoai* diverging in the karyotypic formula, location of the NOR carrier pair, and additional 5S rDNA sites, may be the result of the restriction of gene flow and due to etology this species, that favor the fixation of minor chromosomal rearrangements in the species. Furthermore, results obtained from the COI gene sequences analysis support further investigations in Ancistrini for the middle Iguaçu region, aiming to expand genetic data with a taxonomic focus within this group. Besides this study bringing for the first time COI sequences for populations of *A. abilhoai*, this is also the first to describe the chromosome structure of *H. fuliginosus*, whose molecular data confirm its taxonomic status and its chromosome structure will be a reference for karyoevolutionary discussion within these genera.

ACKNOWLEDGMENTS

Universidade Estadual de Maringá (UEM) and Universidade Estadual do Paraná (UNESPAR), União da Vitória campus, for the logistic and experimental support. To Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq, for the financial support (process number 140704/2018-3).

REFERENCES

- **Armbruster JW, Werneke DC, Tan M.** Three new species of saddled Loricariid catfishes, and a review of *Hemiancistrus*, *Peckoltia*, and allied genera (Siluriformes). ZooKeys. 2015; 480:97–123. https://doi.org/10.3897/zookeys.480.6540
- **Artoni RF, Bertollo LA.** Trends in the karyotype evolution of Loricariidae fish (Siluriformes). Hereditas. 2001; 134(3):201–10. https://doi.org/10.1111/j.1601-5223.2001.00201.x
- **Alves AL, Oliveira C, Nirchio M, Granado A, Foresti F.** Karyotypic relationships among the tribes of Hypostominae (Siluriformes: Loricariidae) with description of XO sex chromosome system in a Neotropical fish species. Genetica. 2006; 128:1–09. https://doi.org/10.1007/s10709-005-0715-1
- **Barros AV, Wolski MAV, Nogaroto V, Almeida MC, Moreira-Filho O, Vicari MR.** Fragile sites, dysfunctional telomere and chromosome fusions: what is 5S rDNA role? Gene. 2017; 608:20–27. https://doi.org/10.1016/j.gene.2017.01.013
- **Baumgartner G, Pavanelli CS, Baumgartner D, Bifi AG, Debona T, Frana VA.** Peixes do baixo rio Iguaçu. Maringá: EDUEM; 2012.
- **Bertollo LAC, Takahashi CS, Moreira-Filho O.** Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Rev Bras Genet. 1978; 1:103–20.
- **Bifi AG, Pavanelli CS, Zawadzki CH.** Three new species of *Ancistrus* Kner, 1854 (Siluriformes: Loricariidae) from the Rio Iguaçu basin, Paraná State, Brazil. Zootaxa. 2009; 2275(1):41–59. https://doi.org/10.11646/zootaxa.2275.1.3
- **Borba RS, Mariotto S, Centofante L, Zawadzki CH, Parise-Maltempi PP.** Molecular discrimination of *Ancistrus* lineages (Siluriformes: Loricariidae) using barcode DNA tool. Mitochondrial DNA Part A. 2019; 30(4). https://doi.org/10.1080/24701394.2019.1597071
- **Bueno V, Konerat JT, Zawadzki CH, Venere PC, Blanco DR, Margarido VP.** Divergent chromosome evolution in Hypostominae Tribes (Siluriformes: Loricariidae): correlation of chromosomal data with morphological and molecular phylogenies. Zebrafish. 2018; 15(5):492–503. https://doi.org/10.1089/zeb.2018.1612
- **Bueno V, Venere PC, Konerat JT, Zawadzki CH, Vicari MR, Margarido VP.** Physical mapping of the 5S and 18S rDNA in ten species of *Hypostomus* Lacépède 1803 (Siluriformes: Loricariidae): Evolutionary tendencies in the genus. Sci World J. 2014; 2014(3):1–08. https://doi.org/10.1155/2014/943825
- **Cardoso AR, Malabarba LR.** Description of three new species of *Hemiancistrus* Bleeker, 1862 from southern Brazil (Teleostei: Siluriformes: Loricariidae). Comun Mus Ciênc Tecnol PUCRS, Sér Zool. 1999; 12:141–61.
- **Cardoso AR, Silva JFP.** Two new species of the genus *Hemiancistrus* Bleeker (Teleostei: Siluriformes: Loricariidae) from the upper rio Uruguai basin. Neotrop Ichthyol. 2004; 2(1):1–08. https://doi.org/10.1590/S1679-62252004000100001
- **Favarato RM, Ribeiro LB, Feldberg E, Matoso DA.** Chromosomal mapping of transposable elements of the rex family in the bristlenose catfish, *Ancistrus* (Siluriformes, Loricariidae), from the Amazonian region. J Heredity. 2017; 108(3):254–61. https://doi.org/10.1093/jhered/esw084
- **Favarato RM, Silva M, Oliveira RR, Artoni RF, Feldberg E, Matoso DA.** Cytogenetic diversity and the evolutionary dynamics of rDNA genes and telomeric sequences in the *Ancistrus* genus (Loricariidae: Ancistrini). Zebrafish. 2016; 13(2):103–11. https://doi.org/10.1089/zeb.2015.1140
- **Fricke R, Eschmeyer WN, Fong JD.** Species by subfamily/ subfamily [Internet]. San Francisco: California Academy of Science; 2023. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp
- **Glugoski L, Deon G, Schott S, Vicari MR, Nogaroto V, Moreira-Filho O.** Comparative cytogenetic analyses in *Ancistrus* species (Siluriformes: Loricariidae). Neotrop Ichthyol. 2020; 18(2):e200013. https://doi.org/10.1590/1982-0224-2020-0013

- **Glugoski L, Giuliano-Caetano L, Moreira-Filho O, Vicari MR, Nogaroto V.** Colocated hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. Gene. 2018; 650:49–54. https://doi.org/10.1016/j.gene.2018.01.099
- **Griffiths SP.** The use clove oil as an anaesthetic and method for sampling intertidal rockpool fishes. J Fish Biol. 2000; 57(6):1453–64. https://doi.org/10.1111/j.1095-8649.2000.tb02224.x
- **Hall TA.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999; 41:95–98. Available from: https://bioedit.software.informer.com/
- **Hatanaka T, Galetti Jr. PM.** Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). Genetica. 2004; 122(3):239–44. https://doi.org/10.1007/s10709-004-2039-y
- **Hebert PDN, Cywinska A, Ball SL, Dewaard JR.** Biological identifications through DNA barcodes. Proc Biol Sci. 2003; 270(1512):313–21. https://doi.org/10.1098/rspb.2002.2218
- **Howell WM, Black DA.** Controlled silverstaining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia. 1980; 36(8):1014–15. https://doi.org/10.1007/bf01953855
- **Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN.** Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes. 2007; 7(4):544–48. https://doi.org/10.1111/j.1471-8286.2007.01748.x
- **Konerat JT, Bueno V, Margarido VP, Portela-Castro ALB, Martins-Santos IC.** Diversity of sex chromosome systems in Ancistrini (Loricariidae, Hypostominae): ZZ/ZW in *Ancistrus taunayi* Miranda Ribeiro, 1918. Cytogenet Genome Res. 2015; 146(4):306–10. https://doi.org/10.1159/000441431
- **Kumar S, Stecher G, Tamura K.** MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016; 33(7):1870–74. https://doi.org/10.1093/molbev/msw054
- **Langeani F, Castro RMC, Oyakawa OT, Shibatta OA, Pavanelli CS, Casatti L.** Diversity of the ichthyofauna of the upper Paraná River: current composition and future prospects. Biota Neotrop. 2007; 7(3):181–97. https://doi.org/10.1590/S1676-06032007000300020
- **Levan A, Fredga K, Sandberg AA.** Nomenclature for centromeric position on chromosomes. Hereditas. 1964; 52(2):201–20. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x
- **Lui RL, Blanco DR, Moreira-Filho O, Margarido VP.** Propidium iodide for making heterochromatin more evident in the C-banding technique. Biotech Histochem. 2012; 87(7):433–38. https://doi.org/10.3109/10520295.2012.696700
- **Lujan NK, Armbruster JW, Lovejoy NR, López-Fernández H.** Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. Mol Phylogenet Evol. 2015; 82:269–88. https://doi.org/10.1016/j.ympev.2014.08.020
- **Lundberg JG.** The temporal context of the diversification of Neotropical fishes. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p.49–68.
- **Mariotto S, Artoni RF, Miyazawa CS.** Occurence of sexual chromosome, of the type ZZ/ZW, in *Ancistrus* cf. *dubius* (Loricariidae: Ancistrinae) of the Paraguay River Basin, Mato Grosso, Brazil. Caryologia. 2004; 57(4):327–31. https://doi.org/10.1080/00087114.2004.10589413
- **Mariotto S, Centofante L, Miyazawa CS, Bertollo LAC, Moreira-Filho O.** Chromosome polymorphism in *Ancistrus cuiabae* Knaack, 1999 (Siluriformes: Loricariidae: Ancistrini). Neotrop Ichthyol. 2009; 7(4):595–600. https://doi.org/10.1590/S1679-62252009000400006
- **Mariotto S, Centofante L, Moreira Filho O.** Diversity and chromosomal evolution in the genus *Ancistrus* Kner, 1854 (Loricariidae: Ancistrini) from three hydrographic basins of Mato Grosso State, Brazil. Neotrop Ichthyol. 2013; 11(1):125–31. https://doi.org/10.1590/S1679-62252013000100015

- **Mariotto S, Centofante L, Vicari MR, Artoni RF, Moreira-Filho O.** Chromosomal diversification in ribosomal DNA sites in *Ancistrus* Kner, 1854 (Loricariidae, Ancistrini) from three hydrographic basins of Mato Grosso, Brazil. Comp Cytogenet. 2011; 5(4):289–300. https://doi.org/10.3897/CompCytogen.v5i4.1757
- **Mariotto S, Miyazawa CS.** *Ancistrus* cf. *dubius* (Siluriformes: Ancistrinae), a complex of species. 1. Chromosomal characterization of four populations and occurrence of sex chromosomes of the type XX/XY, in the Pantanal Basin of Mato Grosso, Brazil. Caryologia. 2006; 59(4):299–304. https://doi.org/10.1080/00087114.2006.10797929
- **Martins C, Galetti Jr. PM.** Chromosomal localization of 5S rDNA genes in *Leporinus* Fish (Anostomidae, Characiformes). Chromosome Res. 1999; 7(5):363–67. https://doi.org/10.1023/a:1009216030316
- **Medeiros LA, Ginani EG, Sousa LM, Rapp Py-Daniel LH, Feldberg E.** Cytogenetic analysis of *Baryancistrus xanthellus* (Siluriformes: Loricariidae: Ancistrini), an ornamental fish endemic to the Xingu River, Brazil. Neotrop Ichthyol. 2016; 14(2):e150108. https://doi:10.1590/1982-0224-20150108
- **Montoya-Burgos JI.** Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. Mol Ecol. 2003; 12(7):1855–67. https://doi:10.1046/j.1365-294X.2003.01857.x
- **de Oliveira RR, Feldberg E, Anjos MB, Zuanon J.** Karyotype characterization and ZZ/ZW sex chromosome heteromorphism in two species of the catfish genus *Ancistrus* Kner, 1854 (Siluriformes: Loricariidae) from the Amazon basin. Neotrop Ichthyol. 2007; 5(3):301–06. https://doi.org/10.1590/S1679-62252007000300010
- **de Oliveira RR, Feldberg E, Anjos MB, Zuanon J.** Occurrence of multiple sexual chromosomes (XX/XY1Y2 and Z1Z1Z2Z2/Z1Z2W1W2) in catfishes of the genus *Ancistrus* (Siluriformes: Loricariidae) from the Amazon basin. Genetica. 2008; 134:243–49. https://doi.org/10.1007/s10709-007-9231-9
- **de Oliveira RR, Feldberg E, Anjos MB, Zuanon J.** Mechanisms of chromosomal evolution and its possible relation to natural history characteristics in *Ancistrus* catfishes (Siluriformes: Loricariidae). J Fish Biol. 2009; 75(9):2209–25. https://doi.org/10.1111/j.1095-8649.2009.02450.x
- **de Oliveira RR, Souza IL, Venere PC.** Karyotype description of three species of Loricariidae (Siluriformes) and occurrence of the ZZ/ZW sexual system in *Hemiancistrus spilomma* Cardoso & Lucinda, 2003. Neotrop Ichthyol. 2006; 4(1):93–97. https://doi.org/10.1590/S1679-62252006000100010
- **Pinkel D, Straume T, Gray JW.** Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA. 1986; 83(9):2934–38. https://doi.org/10.1073/pnas.83.9.2934
- **Primo CC, Glugoski L, Almeida MC, Zawadzki CH, Moreira-Filho O, Vicari MR, Nogaroto V.** Mechanisms of chromosomal diversification in species of *Rineloricaria* (Actinopterygii: Siluriformes: Loricariidae). Zebrafish. 2017; 14(2):161–68. https://doi.org/10.1089/zeb.2016.1386
- **Prizon AC, Borin-Carvalho LA, Bruschi DP, Ribeiro MO, Barbosa LM, Ferreira GEB, Cius A, Zawadzki CH, Portela-Castro ALB.** Cytogenetic data on *Ancistrus* sp. (Siluriformes, Loricariidae) of the Paraguay River basin (MS) sheds light on intrageneric karyotype diversification. Comp Cytogenet. 2016; 10(4):625–36. https://doi.org/10.3897/CompCytogen.v10i4.8532
- **Prizon AC, Bruschi DP, Borin-Carvalho LA, Cius A, Barbosa LM, Ruiz HB, Zawadzki CH, Fenocchio AS, Portela-Castro ALB.** Hidden diversity in the populations of the armored catfish *Ancistrus* Kner, 1854 (Loricariidae, Hypostominae) from the Paraná River basin revealed by molecular and cytogenetic data. Front Genet. 2017; 8:185. https://doi.org/10.3389/fgene.2017.00185
- **Prizon AC, Bruschi DP, Gazolla CB, Borin-Carvalho LA, Portela-Castro ALB.** Chromosome spreading of the retrotransposable Rex-3 element and microsatellite repeats in karyotypes of the *Ancistrus* populations. Zebrafish. 2018; 15(5):1–11. https://doi.org/10.1089/zeb.2018.1620

- **Reis DAR, Brandão KO, Toledo LFA, Pazza R, Kavalco KF.** Localização física dos genes ribossomais 5S e 18S em *Ancistrus* sp. (Loricariidae: Ancistrini) de Angra dos Reis/RJ, bacia dos rios costeiros. Evol Conserv Biodivers. 2012; 3:39–44.
- **Ribeiro MO, Noleto RB, Lorscheider ACA, Porto FE, Prizon AC, Zawadzki CH, Oliveira LC, Portela-Castro ALB.** Cytogenetic description of *Ancistrus abilhoai* (Siluriformes: Loricariidae) from Iguaçu River basin, southern Brazil. Genet Mol Res. 2015; 14(2):4051–57. https://doi.org/10.4238/2015.April.27.20
- **Rocchi M, Archidiacono N, Schempp W, Capozzi O, Stanyon R.** Centromere repositioning in mammals. Heredity. 2012; 108(1):59–67. https://doi.org/10.1038/hdy.2011.101
- **Rosa KO, Ziemniczak K, Barros AV, Nogaroto V, Almeida MC, Cestari MM, Artoni RF, Vicari MR.** Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): fusion points carrying 5S rDNA or telomere sequence vestiges. Rev Fish Biol Fish. 2012; 22:739–49. https://doi.org/10.1007/s11160-011-9250-6
- **Rosenthal A, Coutelle O, Craxton M.** Large-scale production of DNA sequencing templates by microtitre format PCR. Nucleic Acids Research. 1993; 21(1):173–74. https://doi.org/10.1093%2Fnar%2F21.1.173
- **Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A.** DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol. 2017; 34(12):3299–302. https://doi.org/10.1093/molbev/msx248
- **Rubert M.** Cytogenetic studies in species of the tribes Hypostomini and Ancistrini (Loricaridae, Hypostominae). [PhD Thesis]. 2011. São Carlos, Universidade Federal de São Carlos.
- **Silva KS, Souza ACP, Pety AM, Noronha RCR, Vicari MR, Pieczarka JC, Nagamachi CY.** Comparative cytogenetics analysis among *Peckoltia* species (Siluriformes, Loricariidae): Insights on karyotype evolution and biogeography in the Amazon region. Front Genet. 2021; 12:779. https://doi.org/10.3389/fgene.2021.779464
- **Sistema de Informações de Recursos Hídricos de Santa Catarina (SIRHESC).** Bacias hidrográficas do Estado. 2021. Available from: http://www.aguas.sc.gov.br/base-documental/bacias-hidrograficas-do-estado
- **Schemberger MO, Nascimento VD, Coan R, Ramos E, Nogaroto V, Ziemniczak K, Valente GT, Moreira-Filho O, Martins C, Vicari MR.** DNA transposon invasion and microsatellite accumulation guide W chromosome differentiation in a Neotropical fish genome. Chromosoma. 2019; 128(4):547–60. https://doi.org/10.1007/s00412-019-00721-9
- **Sumner AT.** A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 1972; 75(1):304–06. https://doi.org/10.1016/0014-4827(72)90558-7
- **Traldi JB, Blanco DR, Vicari MR, Martinez JF, Lui RL, Barros AV, Artoni RF, Moreira-Filho O.** Chromosomal diversity in *Hypostomus* (Siluriformes, Loricariidae) with emphasis on physical mapping of 18S and 5S rDNA sites. Genet Mol Res. 2013; 12(1):463–71. https://doi.org/10.4238/2013.february.8.11
- **Vari RP, Malabarba LR.** Neotropical ichthyology: an overview. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p.1–11.
- **Waugh J.** DNA barcoding in animal species: progress, potential and pitfalls. Bioessays. 2007; 29(2):188–197. https://doi.org/10.1002/bies.20529
- **Ziemniczak K, Barros AV, Rosa KO, Nogaroto V, Almeida MC, Cestari MM, Moreira-Filho O, Artoni RF, Vicari MR.** Comparative cytogenetics of Loricariidae (Actinopterygii: Siluriformes): emphasis in Neoplecostominae and Hypoptopomatinae. Ital J Zool. 2012; 79(4):492–501. https://doi.org/10.1080/11250003.2012.676677

AUTHORS' CONTRIBUTION

Marcos Otávio Ribeiro: Conceptualization, Investigation, Methodology, Resources, Writing-original draft.

Isabelle Pereira Mari Ribeiro: Resources, Software.

Diego Mauro Carneiro Pereira: Resources.

Thais Aparecida Dulz: Investigation.

Claudio Henrique Zawadzki: Supervision.

Rafael Bueno Noleto: Resources.

Carla Andreia Lorscheider: Resources.

Alessandra Valéria de Oliveira: Resources.

Ana Luiza de Brito Portela Castro: Resources, Supervision, Validation, Writing-review and editing.

ETHICAL STATEMENT

The capture of the specimens was authorized by the Ministério do Meio Ambiente, through the Sistema de Autorização e Informação em Biodibervidade (SISBIO) license number 68533–1. Access to the genetic heritage of the species was authorized by the Sistema Nacional de Gestão do Patrimônio Genético (SISGEN), according to registration nº AAAD7D9. The procedure of euthanasia of the specimens realized in this study, was authorized by the ethics committee of the Universidade Estadual do Paraná (CEUA number 002–2021).

COMPETING INTERESTS

The author declares no competing interests.

HOW TO CITE THIS ARTICLE

- **Ribeiro MO, Ribeiro IPM, Pereira DMC, Dulz TA, Zawadzki CH, Noleto RB, Lorscheider CA, Oliveira AV, Castro ALBP.** Cytogenetic and molecular studies in species of the Ancistrini tribe from Southern Brazil. Neotrop Ichthyol. 2024; 22(1):e220118. https://doi.org/10.1590/1982-0224-2022-0118

Neotropical Ichthyology

OPEN ACCESS

Official Journal of the
Sociedade Brasileira de Ictiologia

SBI