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Karyotype uniformity in populations of the endemic *Hemiancistrus fuliginosus* (Loricariidae: Hypostomini) collected in the upper and middle Uruguai River

Jocicléia Thums Konerat[1], Vanessa Bueno[2], Ana Luiza de Brito Portela-Castro[3], Isabel Cristina Martins-Santos[3] and Vladimir Pavan Margarido[1]

Correspondence:
Vladimir Pavan Margarido
vladimir.margarido@unioeste.br

Disagreements in molecular and morphological analyzes have generated conflicts about the correct allocation of *Hemiancistrus fuliginosus* in Hypostominae. In this study, cytogenetics analyzes in four populations of *H. fuliginosus* from tributaries of the Uruguai River revealed 2n = 56 chromosomes (30m + 18sm + 6st + 2a) for all populations. Nucleolar organizer regions (NORs) were located on the short arm in terminal position of the subtelocentric chromosome pair 25 in all population, in addition to Antas River population showed a structural polymorphism (three different phenotypes). Physical mapping of 5S rDNA showed cistrons in pericentromeric position on the short arm of the metacentric chromosome pair 12 in all the populations. Centromeric heterochromatins are present in almost all chromosomes, and conspicuous CMA_3^+/$DAPI^-$ blocks coincident with rDNA sites. Chromosomal data were important markers to fill gaps and to contribute to morphological and molecular proposals in allocating *H. fuliginosus*. The exclusivity of NORs polymorphism of the Antas River population can be attributed to the geomorphological characteristics of the tributary that restrict gene flow, while karyotypic similarities among the other three populations would be provided by the species' ability to disperse.

Keywords: Chromosomal evolution, Karyotypic similarities, Paracentric inversion, rDNA-FISH.

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1 Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Rua Universitária, 2069, 85819-110 Cascavel, PR, Brazil. (JTK) jocicleia.konerat@unioeste.br, (VPM) vladimir.margarido@unioeste.br (corresponding author).

2 Coordenação do Curso de Licenciatura em Ciências Biológicas, Universidade Tecnológica Federal do Paraná, Campus Santa Helena. Prolongamento da Rua Cerejeira, s/n, 85892-000 Santa Helena, PR, Brazil. (VB) vanessab@utfpr.edu.br.

3 Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Avenida Colombo, 5790, 87020-900 Maringá, PR, Brazil. (ALBPC) albpcastro@nupelia.uem.br, (ICMS) icmdsantos@uem.br.

Divergências nas análises moleculares e morfológicas têm gerado conflitos sobre a correta alocação de *Hemiancistrus fuliginosus* em Hypostominae. Neste estudo, análises citogenéticas em quatro populações de *H. fuliginosus* de afluentes do rio Uruguai revelaram 2n = 56 cromossomos (30m + 18sm + 6st + 2a) para todas as populações. As regiões organizadoras nucleolares (RONs) foram localizadas no braço curto em posição terminal do par cromossômico subtelocêntrico 25 em todas as populações, além da população do rio Antas apresentar polimorfismo estrutural (três fenótipos diferentes). O mapeamento físico do DNAr 5S mostrou cistrons em posição pericentromérica no braço curto do par cromossômico metacêntrico 12 em todas as populações. A heterocromatina centromérica está presente em quase todos os cromossomos e blocos conspícuos de CMA_3^+/$DAPI^-$ coincidentes com sítios de DNAr. Os dados cromossômicos foram marcadores importantes para preencher lacunas e contribuir para propostas morfológicas e moleculares na alocação de *H. fuliginosus*. A exclusividade do polimorfismo das RONs da população do rio Antas pode ser atribuído às características geomorfológicas do tributário que restringem o fluxo gênico, enquanto as semelhanças cariotípicas entre as outras três populações seriam proporcionadas pela capacidade de dispersão da espécie.

Palavras-chave: DNAr-FISH, Evolução cromossômica, Inversão paracêntrica, Semelhanças cariotípicas.

INTRODUCTION

The Uruguai River basin comprises an area of approximately 365,000 km^2, of which 176,000 km^2 are located in Brazilian territory. The Uruguai River is formed by the confluence of the Pelotas and Canoas Rivers, and is subdivided into three regions: upper Uruguai River, middle Uruguai River, and lower Uruguai River (Zaniboni-Filho, Schulz, 2003). The Yucumã waterfall, an important longitudinal waterfall that extends over 1.8 km along the Uruguai River, delimits the upper Uruguai River from the middle Uruguai River (Zaniboni-Filho, Schulz, 2003; Abell *et al*., 2008), while the Salto Grande is the boundary between the middle and lower Uruguai River (Zaniboni-Filho, Schulz, 2003). The Uruguai River, in Brazilian territory, flows over the basalts of the Serra Geral Formation to the triple border with Uruguay and Argentina (Latrubesse *et al*., 2005). Hahn, Camara (2000) recorded approximately 250 species of fish in the Uruguai River basin. Through an inventory of the Uruguai River basin in Brazilian territory, Bertaco *et al*. (2016) point out 275 species, 25 being described for the first time, with a record of 78 endemic species, some belonging to the critically endangered, endangered, or vulnerable categories.

Loricariidae presents several taxonomic problems, being a specious family with 1,064 species (Armbruster *et al*., 2015; Lujan *et al*., 2015; Fricke *et al*., 2024) allocated into six subfamilies according to morphological characters (Armbruster, 2004; Reis *et al*., 2006), and with changes in subfamily status for some groups by molecular data (Lujan *et al*., 2015). The molecular studies by Lujan *et al*. (2015) reorganized Hypostominae into

nine tribes or clades (six clades and three tribes), with several Ancistrini species relocated to different clades, data corroborated by molecular analyzes of the high-throughput sequencing of ultraconserved elements (UCES) made by Roxo *et al*. (2019).

Due to the morphological plasticity of loricariids, Armbruster *et al*. (2015) used molecular phylogeny (Lujan *et al*., 2015) and collated with morphological data to review the genera *Hemiancistrus* Bleeker, 1862 and *Peckoltia* Miranda Ribeiro, 1912; these results partially diverge from exclusively morphological (Armbruster, 2004) or molecular (Lujan *et al*., 2015) studies.

Armbruster *et al*. (2015) maintain several species without reliable morphological characters in Ancistrini, recognizing three groups. In '*Hemiancistrus*' *chlorostictus* Cardoso & Malabarba, 1999, species from southern Brazil and Uruguai are allocated until their relationships can be better examined. For *Hemiancistrus*, only the type-species *Hemiancistrus medians* (Kner, 1854) is considered valid (Armbruster *et al*., 2015), with the other species distributed to other clades/groups in Ancistrini or Hypostomini. In the absence of consensus between morphological and molecular analyzes (Armbruster *et al*., 2015; Lujan *et al*., 2015; Roxo *et al*., 2019), such divergences could be elucidated using other markers to resolve this conflict. Cytogenetic analysis in *H. fuliginosus* Cardoso & Malabarba, 1999, together with data from published molecular and morphological phylogenetic analyses, is proposed to try to fill gaps in the chromosomal evolution of the subfamily.

MATERIAL AND METHODS

Sampling sites. Specimens of *H. fuliginosus* were collected from tributaries of the upper and middle Uruguai River and deposited in the Ichthyological Collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia), Universidade Estadual de Maringá (NUP): 8 specimens (6 females and 2 males, NUP 14826) from the Cascalho Stream (Mondaí, SC – 27°02'48.2"S 53°25'17.8"W), 15 specimens (6 females and 9 males, NUP 14828) from the Antas River (Mondaí, SC –27°05'17.3"S 53°23'40.2"W), 13 specimens (2 females and 11 males, NUP 15021) from the Potiribu River (Ijuí, RS – 28°20'31.3"S 53°53'34.6"W) and 41 specimens (24 females and 17 males, NUP 14827) of the Ijuí River (Ijuí, RS – 28°18'06.3"S 53°53'33.6"W), with Cascalho Stream and Antas River belonging to the upper Uruguai River, and Potiribu River and Ijuí River to the middle Uruguai River (Fig. 1).

Cytogenetic analyses. The animals were anesthetized and euthanized by clove oil overdose (Griffths, 2000). Metaphase cells were obtained using the technique proposed by Bertollo *et al*. (1978). According to Levan *et al*. (1964), chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a). NORs were evidenced by silver impregnation according to the technique described by Howell, Black (1980). Heterochromatin was located by C-banding as proposed by Sumner (1972), with modifications suggested by Lui *et al*. (2012). Fluorochrome staining with Chromomycin A_3 (CMA_3) and 4', 6-diamidino-2-phenylindole (DAPI) followed the protocol described by Schweizer (1980). Physical mapping of the 5S rDNA and 18S rDNA sequences was performed by fluorescent *in situ* hybridization (FISH) according to

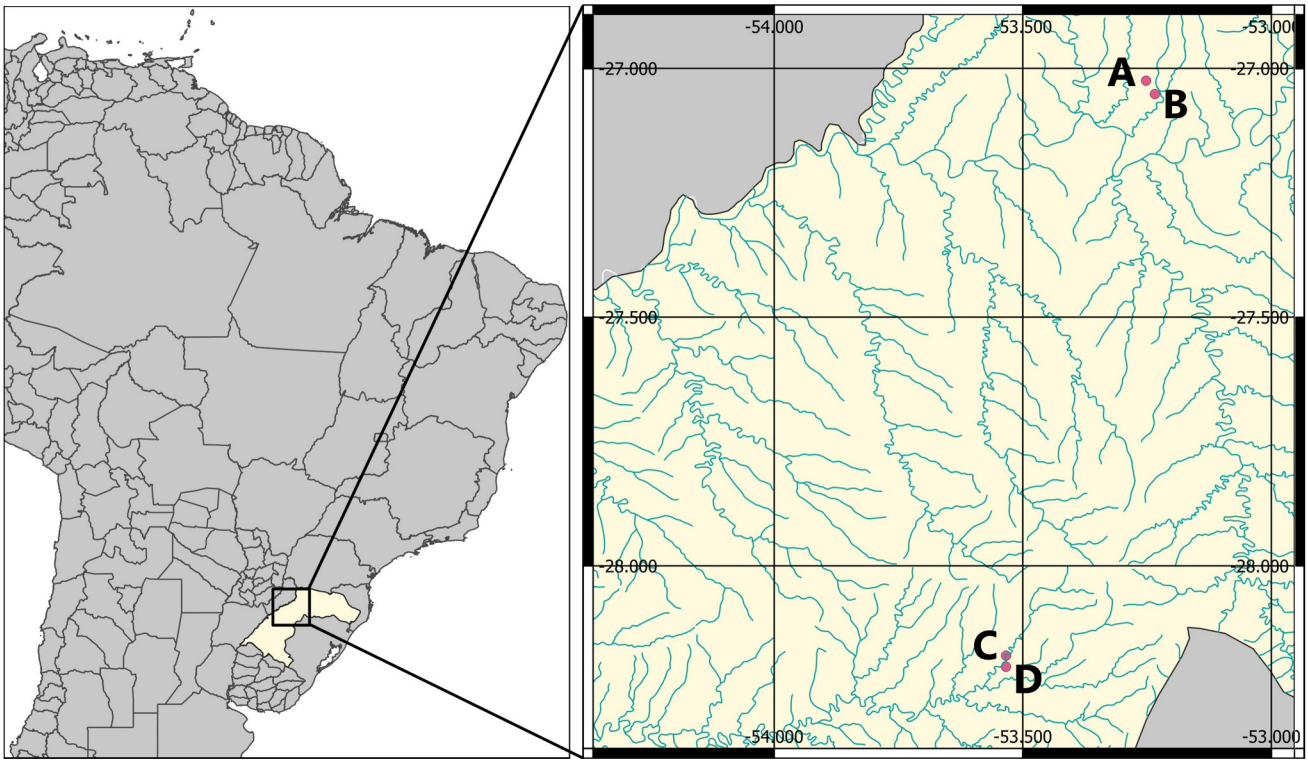

FIGURE 1 | Collection sites of *Hemiancistrus fuliginosus* in the tributaries of the Uruguai River: upper Uruguai River: **A.** Cascalho Stream and **B.** Antas River; middle Uruguai River: **C.** Ijuí River and **D.** Potiribu River.

Pinkel *et al*. (1986) with modifications suggested by Margarido, Moreira-Filho (2008), with DNA probes obtained from *Megaleporinus elongatus* (Valenciennes, 1850) (Martins, Galetti Jr., 1999) and *Prochilodus argenteus* Spix & Agassiz, 1829 (Hatanaka, Galetti Jr., 2004), respectively. Probes were labeled by nick translation with digoxigenin-11-dUTP (5S rDNA) and biotin-16-dUTP (18S rDNA) (Roche®). Signal detection was performed with antidigoxigenin-rhodamine (Roche®) for the 5S rDNA probe and avidin-FITC amplified with biotinylated anti-avidin (Sigma – Aldrich) for the 18S rDNA probe, and the chromosomes were subsequently counterstained with DAPI (50 µg/mL). Metaphases were photographed using the BX 61 epifluorescence microscope and an Olympus DP 71 digital camera with the DP Controller 3.2.1.276 software.

RESULTS

The four populations of *H. fuliginosus* presented 2n = 56 chromosomes (30m + 18sm + 6st + 2a), with no differences between males and females (Fig. 2). Simple NORs (AgNORs and 18S rDNA-FISH) were observed for all analyzed populations, located on the short arm of the subtelocentric chromosome pair 25. In the populations of the Cascalho Stream, Potiribu River, Ijuí River and Antas River, the NORs were located in terminal position (Fig. 2). Heterochromatin was evidenced in the centromeric position of almost all chromosomes, being more evident coincident with NORs, in

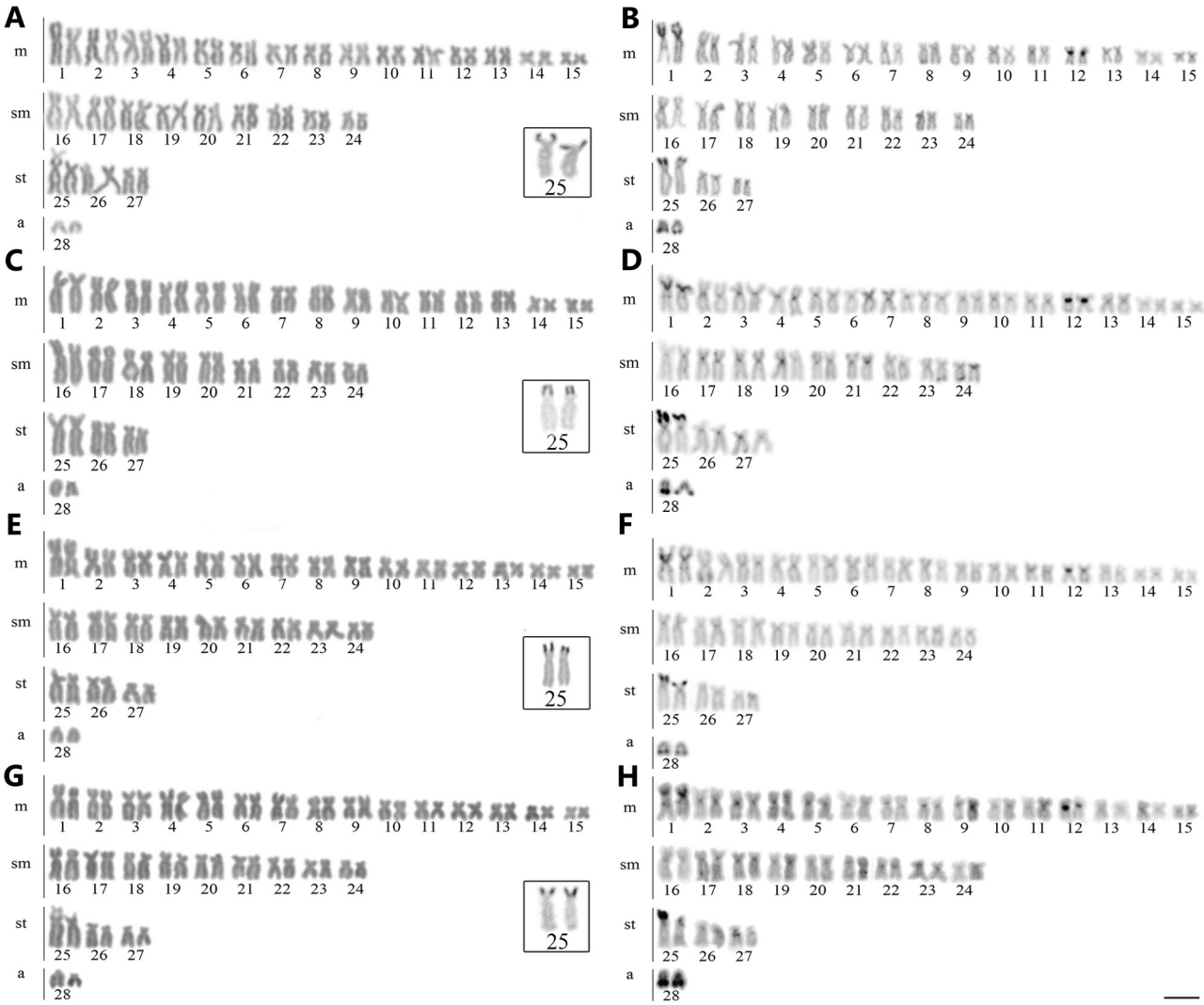

FIGURE 2 | Karyotypes of *Hemiancistrus fuliginosus* stained by Giemsa (**A**–**D**) and C-banded (**E**–**H**). AgNORs are highlighted in the boxes. Cascalho Stream (**A** and **E**), Ijuí River (**B** and **F**), Antas River (**C** and **G**) and Potiribu River (**D** and **H**). Scale bar = 5µm.

the interstitial position of the long arm and pericentromeric position of the short arm of the metacentric chromosome pair 1, in the terminal position of the long arm of the acrocentric chromosome pair 28 and the pericentromeric position of the short arm of the metacentric chromosome pair 12 for the four populations analyzed (Fig. 2). Regarding the 5S rDNA, sites were detected only in the pericentromeric position on the short arm of the 12 metacentric chromosome pair in the four populations analyzed (Fig. 3). Analysis by base-specific fluorochromes showed that the heterochromatin associated with the rDNA regions are $CMA_3^+/DAPI^-$ in all analyzed populations (Fig. 4).The population of Antas River showed polymorphism in the location of NORs sites, with three different phenotypes being observed: seven individuals had both chromosomes carrying terminal NORs (t/t); seven individuals had one chromosome with interstitial NORs and the other chromosome of the pair with terminal NORs (i/t), and one individual had both chromosomes with interstitial NORs (i/i) (Fig. 4). This polymorphism lies in Hardy-Weinberg Equilibrium ($\chi^2 = 0.186$; $0.95 > p > 0.90$; Tab. 1).

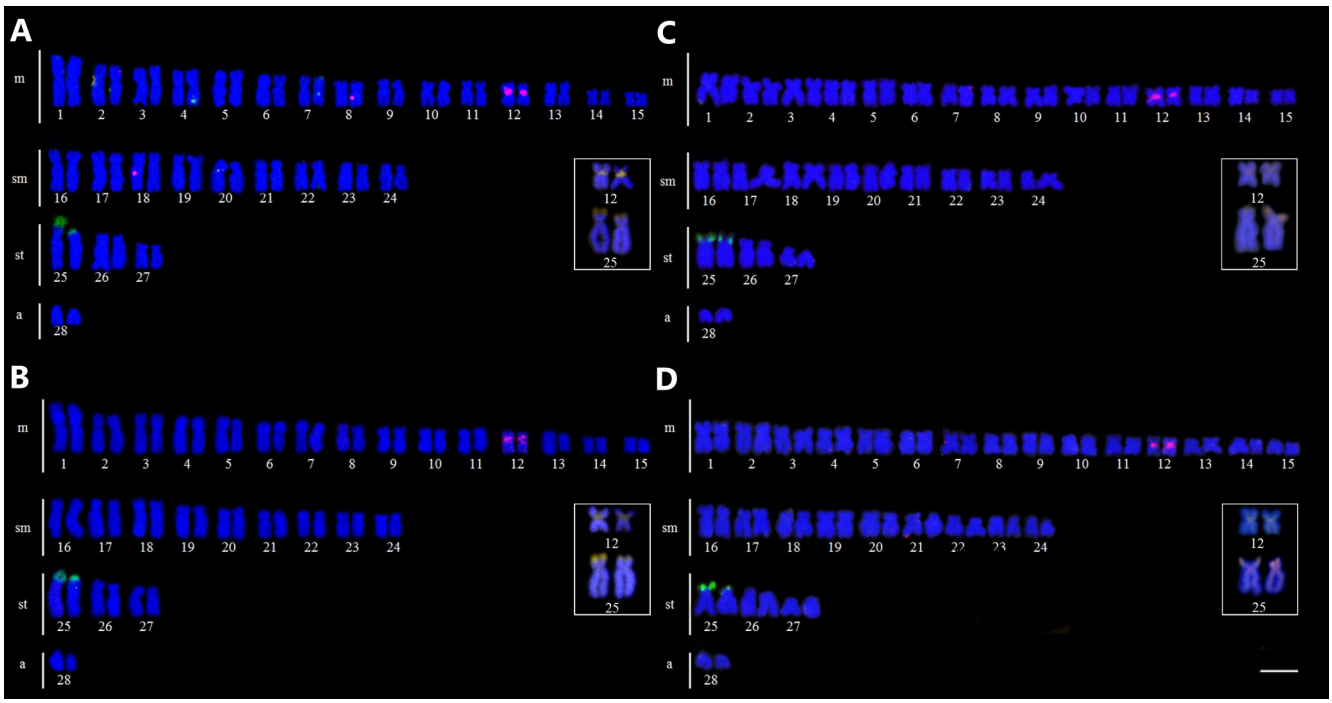

FIGURE 3 | Karyotypes of *Hemiancistrus fuliginosus* submitted to fluorescent *in situ* hybridization with 5S (red) and 18S (green) rDNA probes, counterstained with DAPI, highlighting chromosome pairs 12 and 25 stained with CMA_3/DAPI: **A.** Cascalho Stream, **B.** Antas River, **C.** Ijuí River and **D.** Potiribu River. Scale bar = 5µm.

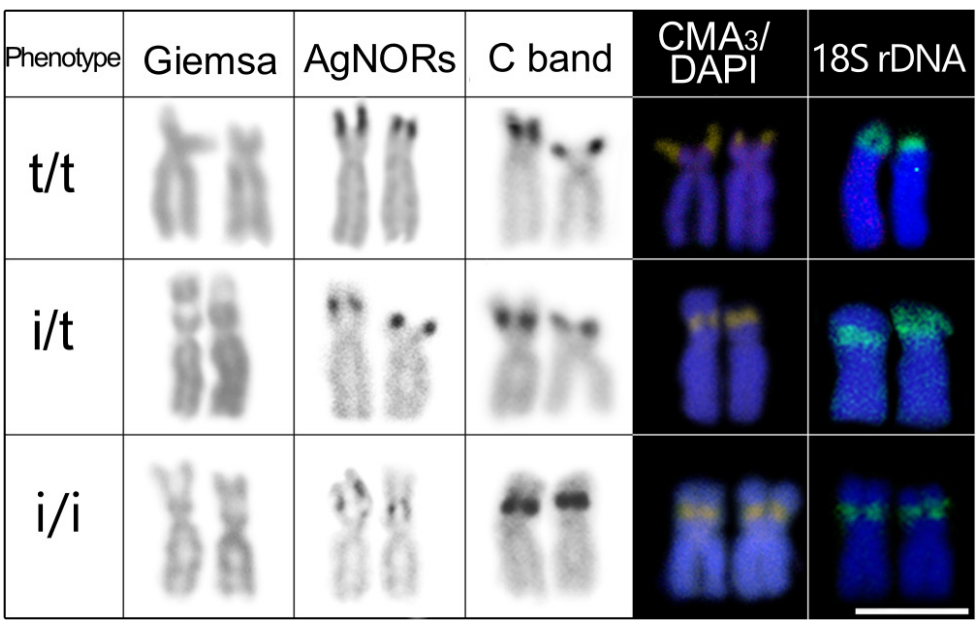

FIGURE 4 | Three phenotypes of the NOR-bearing pair (pair 25) observed in *Hemiancistrus fuliginosus* from Antas River: stained by Giemsa, Ag-staining, C-banded, stained with CMA_3/DAPI and subjected to fluorescent *in situ* hybridization with rDNA probes 18th patterns: terminal/terminal (t/t), interstitial/terminal (i/t) and interstitial/interstitial (i/i). Scale bar = 5µm.

TABLE 1 | Chi-Square test for the three phenotypes of the NOR-bearing pair (pair 25) observed in *Hemiancistrus fuliginosus* from Antas River. O = observed; E = expected; t/t = terminal/terminal; i/t = interstitial/terminal; i/i = interstitial/interstitial.

Phenotype	O	E	$(O-E)^2/E$
t/t	7	7.35	0.017
i/t	7	6.30	0.078
i/i	1	1.35	0.091
Total	15	15	$\chi^2 = 0.186$

DISCUSSION

The phylogenetic relationships within Hypostominae have been debated by different authors, who used different tools (morphological and molecular analyses) to elaborate different proposals (Armbruster *et al*., 2004, 2015; Lujan *et al*., 2015; Roxo *et al*., 2019). Among the existing divergences that appeared among the proposed phylogenies, some are related to the relocation of Ancistrini and Pterygoplichthini species. While cytogenetic analyses provide too little characters to allow the elaboration of a new phylogenetic proposal, the existent phylogenies offer clues to better understand the mechanisms that occurred in the chromosome evolution.

Hemiancistrus has 12 described species (Fricke *et al*., 2024). According to Armbruster *et al.* (2015), *Hemiancistrus* likely contains only the type-species *Hemiancistrus medians*, and new genera would be required to allocate other species groups that are currently allocated in this genus, with *H. fuliginosus* considered a member of the '*Hemiancistrus*' *chlorostictus* group in Ancistrini (Armbruster *et al*., 2015). Morphological studies show that *H. fuliginosus* belongs to *Hemiancistrus*, basal in Ancistrini (Armbruster, 2004), while the molecular data allocate it in Hypostomini (Lujan *et al*., 2015). Morphological studies carried out by Provenzano, Barriga (2017) using the type species *Hemiancistrus medians* for the description and revision of species, show divergences in identifying species already described in *Hemiancistrus*. The authors also suggest revision based on the similarities between *Hemiancistrus* and the representatives of the Hypostomini tribe.

Due to the discrepancy between the morphological and molecular data presented in Hypostominae, it is interesting to use other markers to better solve this conflict. Similar problems were elucidated by including cytogenetic markers in the analyzes (Artoni, Bertollo, 2001; Bueno *et al*., 2012, 2014; Konerat *et al*., 2014a).

Chromosomal evolution in Ancistrini and Hypostomini presents different mechanisms. Ancistrini shows a reduction in the number of chromosomes (Alves *et al*., 2003), with records of 34 to 54 chromosomes (Mariotto *et al*., 2011), while Hypostomini shows an increase in the number of chromosomes (Artoni, Bertollo, 2001), ranging from 54 to 84 chromosomes (Muramoto *et al*., 1968; Cereali *et al*., 2008). Cytogenetic studies in *Pterygoplichthys* Gill, 1858 and *Hemiancistrus* species with divergent allocation show 2n = 52 chromosomes in *Pterygoplichthys* (Alves *et al*., 2006; Fernandes *et al*., 2015; Bueno *et al*., 2018) and *Hemiancistrus* (Artoni, Bertollo, 2001; Oliveira *et al*., 2006), except for *H. fuliginosus* with 2n = 56 chromosomes (Ribeiro *et al*., 2024).

Analyzing the morphological proposals (Armbruster, 2004) with the molecular proposal of Lujan *et al*. (2015) and Roxo *et al*. (2019), in combination with the chromosomal data of Hypostomini and Ancistrini, it is possible to verify divergences of the proposals regarding the positioning of *H. fuliginosus* in Ancistrini. The allocation of *H. fuliginosus* in Hypostomini, as proposed by Lujan *et al*. (2015) and de Roxo *et al*. (2019) is reinforced by the pattern of chromosomal evolution. It is interesting to note that Armbruster *et al.* (2015) also mention that the molecular phylogeny currently offers the bast case for handling this group.

The molecular analysis by Lujan *et al*. (2015) relocates some *Hemiancistrus* species to Hypostomini. The authors proposed *Pterygoplichthys* occupying a basal position, followed by *Hemiancistrus* and *Hypostomus* Lacepède, 1803. In this molecular analysis, *Hemiancistrus* species are allocated in three distinct clades: the '*Peckoltia*' clade, with *Hemiancistrus landoni* Eigenmann, 1916; the '*Hemiancistrus*' clade, containing the genera *Baryancistrus* Rapp Py-Daniel, 1989, *Hemiancistrus*, *Spectrachanticus* Nijssen & Isbrücker, 1987 (cited as *Oligancistrus*), *Parancistrus* Bleeker, 1862 and *Panaque* Eigenmann & Eigenmann, 1889; and the clade corresponding to the tribe Hypostomini, also composed of *Pterygoplichthys* and *Hypostomus*, which contains species of *Hemiancistrus* found in northern South America and restricted to the Uruguai River basin (*i.e*., *H. fuliginosus* and *H. votouro* Cardoso & da Silva, 2004) (Lujan *et al*., 2015). A study based on molecular analyzes on nuclear and mitochondrial genes performed in *Hypostomus* also suggest this position occupied by *Pterygoplichtys* and *Hemiancistrus* in Hypostomini (Cardoso *et al*., 2012).

The molecular studies by Roxo *et al*. (2019) using the UCES resulted in a clade for Loricariidae, with Hypostomini showing the genera *Pterygoplichthys*, *Hypostomus*, and three species of '*Hemiancistrus*' ('*H.*' *fuliginosus*, '*H.*' *punctulatus* Cardoso & Malabarba, 1999 and '*H.*' *cerrado* de Souza, Melo, Chamon & Armbruster, 2008). Using the proposal by Roxo *et al*. (2019), in association with cytogenetic data of the species allocated in Hypostomini, the basal diploid number would change from 54 chromosomes (Muramoto *et al*., 1968) to 52 chromosomes in *Pterygoplichthys* (Fernandes *et al*., 2015; Bueno *et al*., 2018), with the highest diploid number of 84 chromosomes in *Hypostomus perdido* Zawadzki, Tencatt & Froehlich, 2014 (Cereali *et al*., 2008). Chromosomal evolution in Hypostomini has been mainly attributed to the increase in the number of chromosomes, that is, chromosomal rearrangements involved are mainly fission-like (Artoni, Bertollo, 2001). Considering the diploid number 2n = 52 chromosomes plesiomorphic for the tribe (Bueno *et al*., 2018) and cytogenetic data from this study, it is possible to suggest the occurrence of centric fissions responsible for the elevation of the diploid number in *H. fuliginosus*. Thus, the mechanisms involved in the increase in diploid number in Hypostomini are also verified in *Hemiancistrus*.

Data concerning heterochromatin distribution are lacking for the *Hemiancistrus* species allocated to Hypostomini. The checked pattern for *H. fuliginosus* shows pale centromeric heterochromatin and the presence of some conspicuous heterochromatic blocks coincident with the rDNA sites (5S and 18S) that show GC-rich nature. The GC-rich nature pattern coincident with rDNAs is also recorded for *Hypostomus* (Bueno, 2014), and it may be a shared trait in Hypostomini.

Physical mapping of 5S and 18S ribosomal genes has been used in Hypostominae as an important cytogenetic tool for evolutionary discussions (Mariotto *et al*., 2011; Baumgartner *et al*., 2014; Bueno *et al*., 2014), with single and multiple 5S and 18S rDNA

cistrons being recorded for Hypostomini (Bueno *et al*., 2014). For *H. fuliginosus*, the first physical mapping data of rDNA are described, with the description of single cistrons of 5S and 18S rDNA in pericentromeric and terminal positions, respectively. Other rDNA data for Hypostomini show that *P. ambrosettii* (Holmberg, 1893) presents single cistrons of 5S and 18S rDNA in synteny (Bueno *et al*., 2018). The cytogenetic data of *P. ambrosettii* (2n = 52 and rDNA single cistrons in synteny, Bueno *et al*., 2018), (2n = 52 and simple AgNORs, Fernandes *et al*., 2015, cited as *P. anisitsi*) and *H. fuliginosus* (2n = 56 and rDNA single and independent cistrons, this study), strengthens the involvement of fissions in the chromosomal evolution of the tribe (Artoni, Bertollo, 2001). These chromosome fissions would have caused an increase in the diploid number (2n = 52 to 56 chromosomes) and may be related to the loss of rDNA synteny observed in *H. fuliginosus*.

The diploid number, the location of the 5S and 18S rDNA single cistrons, and the nature of the heterochromatin coincident with the rDNA sites of *H. fuliginosus* (this study) associated with those of *P. ambrosettii* (Bueno *et al*., 2018) contribute to filling the gap of cytogenetic data in Hypostomini. These markers corroborate the events already proposed for chromosomal evolution (Artoni, Bertollo, 2001) as well as the proposal of Lujan *et al*. (2015) on the allocation of *H. fuliginosus* in Hypostomini.

For the four populations of *H. fuliginosus*, single NORs (Ag- and 18S rDNA-FISH) were observed. However, the population from Antas River showed a polymorphism concerning the location of these sites, with three different phenotypes: terminal/terminal (t/t), interstitial/terminal (i/t), and interstitial/interstitial (i/i). This polymorphism can be attributed to the occurrence of a paracentric inversion. Possibly for the population of *H. fuliginosus* from Antas River, the presence of heterochromatin coinciding with these regions would have facilitated the occurrence of the paracentric inversion that originated the variant phenotypes. An essential tool to understand the mechanisms involved in the evolutionary process of the fish group is to verify the composition and distribution pattern of heterochromatin, as it has been suggested that heterochromatin plays a relevant role in the occurrence of chromosomal rearrangements (Souza *et al*., 1996; Molina, Galetti, 2002; Konerat *et al*., 2014a,b; Bueno *et al*., 2018).

The number of chromosomes and karyotypic formula found for *H. fuliginosus* coincides with the four populations analyzed; however, the occurrence of NORs polymorphism is exclusive in the Antas River population, suggesting isolation of this population from the others. Although terminal NORs (t) occur more frequently in this population [f(t) = 0.7], the occurrence of Hardy-Weinberg Equilibrium (Tab. 1) suggests a neutral effect of these cytotypes and the absence of genetic flow with the other populations, probably due to the geomorphological characteristics of the Antas River, such as its slope and alternation of low to high depth stretches. The results also suggest that the Uruguai River does not constitute a barrier that could restrict the gene flow between the other populations of the upper and middle Uruguai River. The upper Uruguai River is steep and has fast waters, with flooding between June and October, although significant annual variations in water levels can be observed. The middle Uruguai River shows an average fall and some rapids, while the lower Uruguai River has a total fall of less than one meter. The Uruguai River regions have considerably different hydrological conditions (Zaniboni-Filho, Schulz, 2003).

Hemiancistrus fuliginosus is endemic to the Uruguai River basin (Miquelarena, López, 2004; Lujan *et al*., 2015). It was also recorded as an accessory or occasional species in the Uruguai River, characterized by the diversity of habitats with a rocky bottom and many rapids (Hahn, 2000; Hahn *et al*., 2011), characteristics that may have allowed contact between populations since they preferentially inhabit lotic environments with rapids and rocky substrates (Agostinho *et al*., 2003). The reproductive biology of *Hemiancistrus*, evidenced by studies with *H. punctulatus*, shows that they occupy fairly varied reproduction sites, however incipient in streams; present total spawning and reproductive peak occurring from November to January, with parental care. They are sedentary, with a preferred depth between 0.5 and 5 m. However, it was observed that young individuals occupy shallow and stream habitats while adults generally explore the river (Luz-Agostinho *et al*., 2010; Hirschmann *et al*., 2011). In this way, species of the genus can be found in different habitats, occasionally or incidentally. During the dry season, the Uruguai River presents a drastic reduction in the volume of water, with the formation of environments characteristic of those inhabited by *H. fuliginosus* in its tributaries. Cytogenetic studies performed by Yano *et al*. (2014) in populations of *Psalidodon paranae* (Eigenmann, 1914), *P. fasciatus* (Cuvier, 1819), and *Astyanax lacustris* (Lütken, 1875) (cited as *A. altiparanae* Garutti & Britski, 2000) from two tributary streams of the São Francisco River (upper Paraná River basin) with 36 km of distance from mouth to mouth, verified karyotypic differences between populations for *P. paranae* and *P. fasciatus*, and absence for *A. altiparanae*. The authors attribute that the São Francisco River channel acted as an ecological barrier for the populations of *P. paranae* and *P. fasciatus*, but not for *A. altiparanae*.

Cytogenetic data obtained from these populations corroborate the allocation of *H. fuliginosus* in Hypostomini, showing an increase in diploid number when compared to *Pterygoplichthys*, but lower diploid numbers than *Hypostomus*. The exclusivity of NORs polymorphism detected in the population of *H. fuliginosus* from Antas River can be explained by the geomorphological characteristics of the river, preventing gene flow between populations. The karyotypic similarities detected between the populations of *H. fuliginosus* from the Cascalho Stream, the Ijuí River, and the Potiribu River can be attributed to the presence of similar habitats existing between the tributaries and the Uruguai River itself in the dry season, not showing full restriction of the interpopulation gene flow.

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AUTHORS' CONTRIBUTION

Jocicléia Thums Konerat: Conceptualization, Investigation, Methodology, Project administration, Writing-original draft, Writing-review and editing.

Vanessa Bueno: Conceptualization, Investigation, Methodology, Project administration, Writing-original draft, Writing-review and editing.

Ana Luiza de Brito Portela-Castro: Writing-original draft, Writing-review and editing.

Isabel Cristina Martins-Santos: Project administration, Writing-original draft, Writing-review and editing.

Vladimir Pavan Margarido: Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, approved by the Committee on the Ethics of Animal Experiments of the Universidade Estadual do Oeste do Paraná (license number: Protocol 13/09 – CEEAAP/Unioeste). Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) authorized the capture of the fish (license number: SISBIO 10522–1).

COMPETING INTERESTS

The author declares no competing interests.

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