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Polymorphism of 5S and 18S ribosomal DNA in a conserved karyotype of the two *Hypostomus strigaticeps* (Siluriformes: Loricariidae) populations from the upper Paraná River basin

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Hypostomus is a remarkable group within Siluriformes due to its high species richness, pronounced morphological and chromosomal variability, which complicates the process of species identification. In this context, the aim of this study was to identify possible interpopulational chromosomal markers that could significantly contribute to our understanding of the differentiation in *H. strigaticeps* of distinct tributaries. Two *H. strigaticeps* populations from the upper Paraná River basin were analyzed using cytogenetic methods. Our results revealed interpopulation variations in *H. strigaticeps*, despite limited sampling of the Ivaí River population, particularly in the number of 18S and 5S rDNA sites. Moreover, the individuals from both populations exhibited the same chromosome count (2n = 72) and fundamental number (FN = 108), as well as identical chromosome morphology and similarities in the distribution of constitutive heterochromatin. The transposable elements (TEs) *Rex*1 and *Rex*3 were found scattered along the chromosomes, with a higher concentration of *Rex*3 elements in pairs carrying rDNA. Our findings provide novel insights into a possible involvement of TEs in the variability of rDNA distribution patterns, contributing to karyotypic diversification in *H. strigaticeps*.

Keywords: Heterochromatin, Karyotype evolution, NORs, Retrotransposons, Transposable elements.

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Hypostomus é um grupo notável dentro dos Siluriformes pelo grande número de espécie, alta variabilidade morfológica e cromossômica, o que dificulta o processo de identificação de suas espécies. Nesse contexto, o objetivo deste estudo foi identificar possíveis marcadores cromossômicos interpopulacionais que poderiam contribuir significativamente para nossa compreensão da diferenciação em *H. strigaticeps* de tributários distintos. Duas populações de *H. strigaticeps* da bacia do alto rio Paraná foram analisadas usando métodos citogenéticos. Nossos resultados revelaram variações interpopulacionais em *H. strigaticeps*, apesar da amostragem limitada da população do Rio Ivaí, particularmente no número de sítios de DNAr 18S e 5S. Além disso, os indivíduos de ambas as populações exibiram a mesma contagem de cromossomos (2n = 72) e número fundamental (FN = 108), bem como morfologia cromossômica idêntica e similaridades na distribuição da heterocromatina constitutiva. Os elementos transponíveis (TEs) *Rex*1 e *Rex*3 foram encontrados espalhados ao longo dos cromossomos, com uma concentração maior de elementos *Rex*3 em pares carregando DNAr. Nossas descobertas fornecem importantes novos insights sobre um possível envolvimento de TEs na variabilidade dos padrões de distribuição de DNAr, contribuindo para a diversificação cariotípica em *H. strigaticeps*.

Palavras-chave: Elementos transponíveis, Evolução do cariótipo, Heterocromatina, NORs, Retrotransposons.

INTRODUCTION

Hypostomus Lacépède, 1803 is a highly diverse genus of Loricariidae, with 161 valid species (Fricke *et al*., 2024). This genus presents a remarkable morphological variability, featuring fish that range in size from small to large, popularly known as plecos or "cascudos" (Weber, 2003; Zawadzki *et al*., 2014; Zanata, Pitanga, 2016). The intraspecific diversity in morphology and color pattern of *Hypostomus* species makes taxonomic identification challenging, necessitating constant taxonomic reviews (Weber, 2003).

As a result, several cytogenetic studies have used the terms sp., aff., and cf., to describe species within *Hypostomus*, leading to an estimate of around 40 species with diploid numbers (2n) described (Rubert *et al*., 2022 in supplementary table). These species exhibit a wide variation in 2n from 64 chromosomes in *H. faveolus* Zawadzki, Birindelli & Lima, 2008 from Taquaralzinho River, upper Araguaia River basin, *H. cochliodon* Kner, 1854 from Iguassu River, Paraná River basin (Bueno *et al*., 2013), and *H. soniae* Hollanda Carvalho & Weber, 2005 from Teles Pires River, Southern Amazon basin (Oliveira *et al*., 2019) to 84 chromosomes in *H. perdido* Zawadzki, Tencatt & Froehlich, 2014 from Perdido River, Paraguay River basin (Cereali *et al*., 2008; Zawadzki *et al*., 2014). The karyotypic diversity of the genus has demonstrated not only variability in 2n but also intra- and interspecific structural chromosomal diversity. This suggests that chromosomal rearrangements, mainly centric fissions, and pericentric inversions, were the possible mechanisms involved in the diversification of this group (Artoni, Bertollo, 1996; Artoni, Bertollo, 2001; Alves *et al*., 2005; Bueno *et al*., 2012).

Among the diverse diploid numbers in *Hypostomus*, *Hypostomus strigaticeps* (Regan, 1908) stands out for its conserved 2n, with 72 chromosomes, in various populations (Fernandes *et al*., 2012; Bueno *et al*., 2013; Baumgärtner *et al*., 2014; Rubert *et al*., 2016). This species, initially thought to be unique in its distribution confined to the Tiete River basin (SP) (Weber, 2003), was described in 1908 (Regan, 1908) from the Piracicaba River. Contrary to initial speculations, Borba *et al*. (2013) suggested based on their phylogenetic analysis of different populations of *H. strigaticeps* from the Paraná River basin using ATP synthase sequences (mitochondrial DNA) that they all belong to the same species, rejecting any claim for cryptic species. Additionally, their phylogeographic analysis concluded that the species distribution extends beyond the Tiete River basin to other subbasins of the upper Paraná River (Borba *et al*., 2013).

In *Hypostomus*, cytogenetic variations are not restricted to diploid numbers and karyotype composition. Interspecific, intraspecific and interpopulation variation in the number of heterochromatic bands and ribosomal DNA (rDNA) sites [which include major (45S) and minor (5S) rDNA clusters] is a common cytogenetic feature in *Hypostomus* (Baumgärtner *et al*., 2014; Bueno *et al*., 2014). These events within this genus highlight the pressing need for further investigation to gain a more comprehensive understanding of these polymorphisms.

Transposable elements (TEs) are categorized as retrotransposons (Class I) or DNA transposons (Class II) based on their transposition mechanism, which involves either an RNA intermediate or DNA, respectively (Wicker *et al*., 2007). The teleosts have the highest diversity of TEs in vertebrates (Chalopin *et al*., 2015; Shao *et al*., 2019; Carducci *et al*., 2020), and TEs have been used as chromosomal markers in fish cytogenetics (Ferreira *et al*., 2011), providing valuable insights into the dispersions of specific DNA fragments along chromosomes. According to Volff *et al.* (1999, 2000, 2001), the teleost genomes contain abundant retroelements *Rex*1, *Rex*3, and *Rex*6 and others transposable elements (Ferreira *et al*., 2011; Glugoski *et al*., 2018). In a recent study, Traldi *et al*. (2019) revealed a wide dispersion of *Rex*1 and *Rex*3 retroelements in both heterochromatic and euchromatic regions in species of *Hypostomus*.

Given that *H. strigaticeps* is a resident of the upper Paraná River basin, the present article compared two populations collected from the Pirapó and Ivaí rivers through cytogenetics analysis. The aim was to identify possible interpopulational chromosomal markers that could significantly contribute to our understanding of the differentiation in *H. strigaticeps* of distinct tributaries.

MATERIAL AND METHODS

Study area and sampling. Fourteen individuals of *H. strigaticeps* were collected from populations of the Paraná River basin: seven males and four females from the Pirapó River (municipality of Maringá, Paraná State; 23º18'15" S 51º53'41"W), and two males and one female from the Ivaí River (municipality of Ivatuba, Paraná State; 23º38'17"S 52º15'22"W).

Voucher specimens were deposited in the Fish Collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUPELIA), Universidade Estadual de Maringá, municipality of Maringá, Paraná State, Brazil, as *Hypostomus strigaticeps* from the Pirapó River (NUP 22964), and the Ivaí River (NUP 22970).

Cytogenetic analysis. Metaphase chromosomes were obtained from anterior kidney cells using the air-drying technique (Bertollo *et al*., 2015). The nucleolus organizer regions (NORs) were detected employing silver nitrate staining (Howell, Black, 1980). Constitutive heterochromatin was determined following the C-banding technique (Sumner, 1972) and stained with propidium iodide (Lui *et al*., 2012).

At least 30 metaphases were analyzed for each individual, and those with better chromosome morphology were used for the karyotype analysis. The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a) according to (Levan *et al*., 1964). The fundamental number (FN) was calculated according to the chromosomal arm numbers (the chromosomes m, sm, and st were considered to contain two arms – and the chromosomes with one arm).

The location of the rDNA sites in the chromosomes was performed by fluorescence *in situ* hybridization (FISH) with modifications (Margarido, Moreira-Filho, 2008; Pinkel *et al*., 1986) using probes from the genome of *Megaleporinus elongatus* (Valenciennes, 1850) (Martins, Galetti Jr., 1999) for 5S rDNA sites and *Prochilodus argenteus* Spix & Agassiz, 1829 (Hatanaka, Galetti, 2004) for 18S rDNA sites. The probes were labeled through nick translation with digoxigenin-11-dUTP (5S rDNA) and biotin-16-dUTP (18S rDNA) (Roche Diagnostics, Mannheim, Germany). Detection and amplification of the hybridization signal were carried out using avidin-FITC and anti-avidin biotin (Sigma- Aldrich, St. Louis, Missouri, United States) for probes labeled with biotin and anti-digoxigenin rhodamine (Roche Diagnostics, Mannheim, Germany) for probes labeled with digoxigenin. Chromosomes were counterstained with 4'-6-diamin-2-phenylindole (DAPI, 50 ìg ml^{-1}, Sigma- Aldrich, St. Louis, Missouri, United States).

Transposable element probes were produced using the primers Rex-3 [Foward (5'- CGGTGAYAAAGGGCAGCCCTG-3') and Reverse (5'-TGGCAGACNGGGGTGGTGGT-3') (Volff, 2006). These nucleotide sequences of the *Rex*-3 element, obtained from the genome of different *Ancistrus* populations, revealed a high degree of similarity to the non-LTR Rex-3 retrotransposon, according to sequences deposited in the online BLAST database and confirmed by the CENSOR (Prizon *et al*., 2018). REX-1 [Foward (5'- TTCTCCAGTGCCTTCAACACC-3') and Reverse (5' - TCCCTCAGCAGAAAGAGTCTGCTC-3') (Volff *et al*., 2000). Amplification was performed using PCR, and the probes were labeled according to the nick translation method using the Anti-digoxigenin-Rhodamine Kit (Roche Diagnostics, Mannheim, Germany). Chromosomes were counterstained with 4'-6-diamin-2-phenylindole (DAPI, 50 ìg ml^{-1}, Sigma- Aldrich, St. Louis, Missouri, United States).

Admittedly, the obtained PCR fragments were not sequenced. Nonetheless, although the probes obtained by PCR may occasionally include nonspecific fragments, the primers used (Volff *et al*., 1999, 2000) are recognized for their high specificity in the amplification of Rex1 and Rex3 elements. The identity of the fragments amplified by these primers has been widely confirmed by sequencing, as demonstrated in several studies (Ferreira *et al*., 2011; Prizon *et al*., 2018; among others), including in *Hypostomus* species (Traldi *et al*., 2019). Additionally, cross-hybridizations with Rex1 and Rex3 probes between different species of Loricariidae showed distribution patterns similar to those observed with species-specific probes, indicating a high nucleotide identity between the species (Ferreira *et al*., 2011).

Conventional and fluorescence chromosome preparations were analysed under a BX 51 epifluorescence microscope (Olympus America, Inc). The images were captured using the DP controller (Media Cybernetics) software and the image composition with Adobe Photoshop CS6.

RESULTS

All the 14 individuals of *H. strigaticeps* from the two collection sites had invariably diploid chromosome number of 72 chromosomes, with the karyotype composed of 10 metacentric, 12 submetacentric, 14 subtelocentric, and 36 acrocentric chromosomes and FN value 108 in both sexes (Figs. 1A, D). Heteromorphic sex chromosomes were not identified.

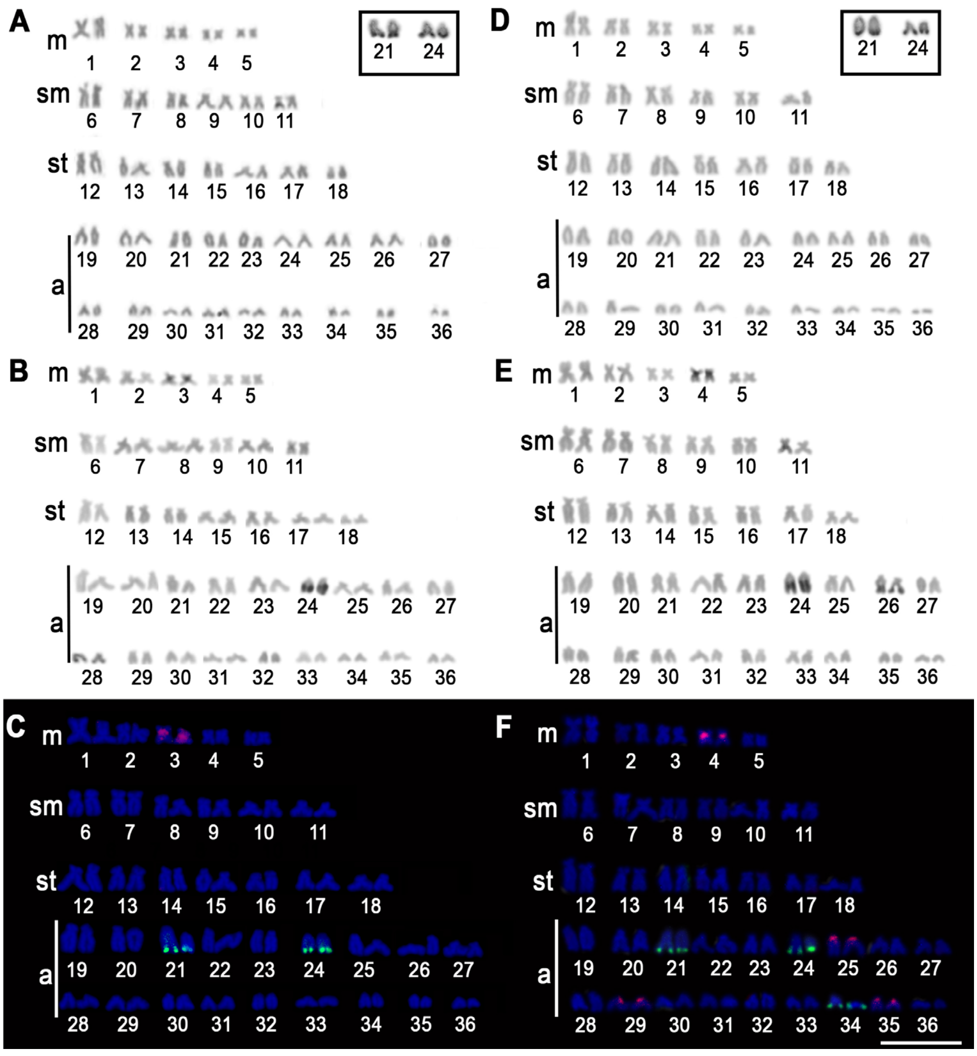

FIGURE 1 | Karyotypes of *Hypostomus strigaticeps* from Pirapó River (**A**, **B**, **C**) and Ivaí River (**D**, **E**, **F**). Karyotypes (**A** and **D**) stained with Giemsa, (**B** and **E**) C-banded, and after double FISH (**C** and **F**) with 5S rDNA probes (red) and 18S rDNA (green). The AgNOR-bearing chromosomes are boxed. Scale bar = 10 µm.

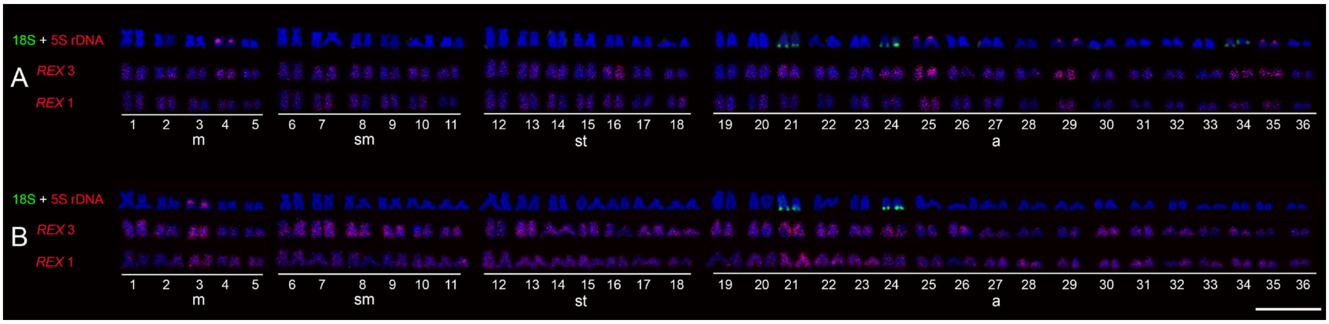

FIGURE 2 | Karyotypes of *Hypostomus strigaticeps* after FISH with rDNA and retroelements *Rex*1 and *Rex3* from Ivaí River (**A**) and Pirapó River (**B**). Scale bar = 10 µm.

The constitutive heterochromatin was located in the pericentromeric region of pairs 3 for Pirapó River population (Fig. 1B) and 4 for Ivaí River population (Fig. 2E), both populations showed large heterochromatic blocks located in terminal position in the long arm of the acrocentric pair 24, and in the same position of the acrocentric pairs 28 for Pirapó River population (Fig. 1B) and 26 for Ivaí River population (Fig. 2E).

The silver nitrate impregnation identified the Ag-NOR in a terminal position of the long arm of the acrocentric pairs 21 and 24 in both populations (Fig. 1, Box). FISH with 18S rDNA probes confirmed the Ag-NOR sites (Figs. 1C, F) and detected further inactive major ribosomal clusters in a terminal position of the long arm of the acrocentric pair 34 for Ivaí River population (Fig. 1F). Moreover, the FISH revealed a single 5S rDNA site in the pericentromeric position of pair 3 for the Pirapó River population. Multiple 5S rDNA sites were observed in the pericentromeric position of pair 4 and the terminal position of the short arm of the acrocentric pairs 25, 29, and 35 in the Ivaí River population (Fig. 1F). None of the 5S rDNA sites co-localized with major rDNA (45S) clusters which have been visualized using the probe for 18S rDNA.

In FISH experiments with transposable elements, we found that the *Rex*1 and *Rex*3 elements are scattered along the chromosomes, including both euchromatic and heterochromatic regions, in all analyzed populations (Fig. 2). This dispersed pattern of Rex1 and Rex3 elements observed in our study is widely described in the literature (including with highly specific probes obtained by cloning and sequencing). Notably, in *H. strigaticeps* from the Ivaí River, there was a higher concentration of *Rex*3 elements in pairs 25, 29, 34, and 35, as well as in one of the homologs of pair 16 (Fig. 2A).

DISCUSSION

Our results revealed interpopulation variations in *H. strigaticeps*, despite limited sampling for the Ivaí River population, particularly in the number of 18S and 5S rDNA sites. Notably, individuals from both populations share the same 2n = 72, FN = 108, identical chromosome morphology, and similarities in the distribution of constitutive heterochromatin. This diploid number of 72 chromosomes is predominant in most populations of *H. strigaticeps*, with the exceptions of populations from Corumbataí River (Alves *et al*., 2012) and the samples from unspecified locality examined by Michele *et* *al*. (1977), which exhibit a diploid number of 2n = 74 (Tab. 1). Considering 2n = 54 chromosomes as basal for Loricariidae (Artoni, Bertollo, 2001), centric fission is likely an important mechanism that contributed to the increase in 2n as observed for *H. strigaticeps* (2n = 72 and 74). Additionally, various populations of *H. strigaticeps* with 2n = 72 exhibit diversification in their karyotype structures (Tab. 1). This suggests chromosome rearrangements, such as pericentric inversions, addition and/or deletions that can alter the chromosome morphology without changing 2n. The geographical isolation of *H. strigaticeps* populations might have played a fundamental role in the establishment of this karyotype variation. The restriction of gene flow between them, as a result of their isolation, could have favored the fixation of such chromosome change. The majority of *Hypostomus* species, including *H. strigaticeps*, are typically formed by small groups of non-migratory animals (Britski *et al*., 1988), suggesting a strong effect of genetic drift.

In our study, the constitutive heterochromatin distribution did not differ significantly between the two populations analyzed. Both populations exhibited pericentromeric markings on a pair of metacentric chromosomes (pair 3 from the Pirapó River and pair 4 from the Ivaí River), coincident with 5S rDNA sites, as well as terminal markings on the long arm of two pairs of acrocentric chromosomes (pairs 24 and 28 from the Pirapó River and pairs 24 and 26 from the Ivaí River). Thus, the C-banding method did not provide any population-specific patterns of heterochromatic segments distribution. Nonetheless, large heterochromatic blocks on the long arm of acrocentric chromosomes identified intra and interpopulation polymorphism in other populations of *H. strigaticeps* (Baumgärtner *et al*., 2014). In other *Hypostomus* species, studied revealed the existence of a conserved pattern of distribution of constitutive heterochromatin. Notably, a

TABLE 1 | Cytogenetic data available in *Hypostomus strigaticeps* from the upper Paraná River basin. ***Various points in the Paranapanema River basin, PR; 2n = diploid number; m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric; NORs = nucleolar organizer regions (number of bearing chromosomes utilizing of Ag-NOR and/or 18S rDNA-FISH); 5S = 5S rDNA cistrons number.

Localities	2n	Karyotype structure	NORs	5S	References
-	74	8m+4sm+62st-a	-	-	Michelle *et al.* (1977)
***	72	10m+16sm+46st-a	4	-	Rubert *et al.* (2011)
Água Boa stream, MS	72	12m+18sm+20st+22a	4	-	Fernandes *et al.* (2012)
Corumbataí River, SP	74	10m+14sm+14st+36a	8	-	Alves *et al.* (2012)
Ivaí River, PR	72	10m+14sm+18st+30a	8	-	Endo *et al.* (2012)
Piquiri River, PR	72	12m+12sm+18st+30a	3	-	Bueno *et al.* (2013)
Hortelã stream, SP	72	10m+18sm+18st+26a	4	12	Pansonato-Alves *et al.* (2013)
Atlântico stream, PR Piquiri River, PR	72	12m+12sm+18st+30a	5		Baumgärtner *et al.* (2014)
Paraná River, PR Iguaçu River, PR			6	6	
Mogi-Guaçu River, SP	72	10m+16sm+46st-a	2	-	Rubert *et al.* (2016)
Pirapó River, PR Ivaí River, PR	72	10m+12sm+14st+36a	4 6	2 8	Present study

pair of metacentric chromosomes exhibits pericentromeric markings (Rubert *et al*., 2011; Traldi *et al*., 2012). Additionally, some st-a chromosomes are characterized by conspicuous blocks located in the terminal regions of the long arms, which may account for approximately one-third to one-half of the chromosomes total lengths (Artoni, Bertollo 1999; Kavalco *et al*., 2004; Cereali *et al*., 2008; Baumgartner *et al*., 2014). Both forms described for these chromosomes are similar to those found in *H. strigaticeps* from the populations analyzed.

Although both populations of *H. strigaticeps* presented the same karyotypic structure and similarities in the pattern of C-bands, they differed in the number of 18S and 5S rDNA sites. The application of 18S rDNA-FISH confirmed the Ag-NORs sites, and detected further inactive major ribosomal clusters for Ivaí River population. Multiple NORs are common in *H. strigaticeps* (Tab. 1), except for the individuals of the Mogi-Guaçu River (Rubert *et al*., 2016). Furthermore, the locations of NORs in acrocentric chromosomes in *H. strigaticeps* coincide with observations in other *Hypostomus* species and generally correspond to regions of constitutive heterochromatin (Kavalco *et al*., 2004; Rubert *et al*., 2008; Traldi *et al*., 2013; Baumgartner *et al*., 2014), such as the pair 24 in both populations in the present study. This association may contribute to the dispersion of NOR sites throughout the genome via transposable elements (TEs), which tend to accumulate in heterochromatic regions and may carry portions of rDNA clusters (Cioffi *et al*., 2010; Symonová *et al*., 2013; Sember *et al*., 2015; Glugoski *et al*., 2018; Yano *et al*., 2020). Chromosomal pairs 21, 24, and 34 of *H strigaticeps* (population from the Ivaí River), carrying Ag-NOR/18S rDNA sites, showed a high concentration of *Rex*3 elements, distributed throughout the entire length of these chromosomes, corroborating the above hypothesis.

The two populations of *Hypostomus strigaticeps* exhibited variability in both the quantity and the spatial distribution of 5S rDNA sequences. These genes, located at the interstitial position of a metacentric chromosome pair, similar to pair 4 (Ivaí River population) and 3 (Pirapó River population) in the present study, have been reported for *H. strigaticeps* (Pansonato-Alves *et al*., 2013; Baumgärtner *et al*., 2014), *H. regani* (Ihering, 1905) (Mendes-Neto *et al*., 2011; Ferreira *et al*., 2019), *H. nigromaculatus* (Schubart, 1964) (Martins, Galetti Jr., 2001), and *H. iheringii* (Regan, 1908) (Traldi *et al*., 2012). In Hypostominae, the 5S rDNA arrays located at the interstitial position of a metacentric chromosome pair also have been reported for *Rhinelepis aspera* Spix & Agassiz, 1829 (Bueno *et al*., 2018), *Ancistrus* populations (Prizon *et al*., 2017), *Hypancistrus* cf. *debilittera* Armbruster, Lujan & Taphorn, 2007, *H. zebra* Isbrüeker & Nijssen, 1991(Silva *et al*., 2014), and *Baryancistrus xanthellus* Rapp Py-Daniel, Zuanon & de Oliveira, 2011 (Medeiros *et al*., 2016).

In contrast, the additional 5S rDNA clusters identified on three pairs of acrocentric chromosomes were found in the terminal position on the short arm in the present study for *H. strigaticeps* from the Ivaí River population. A hypothesis that may explain the increased dispersion of 5S rDNA gene copies in this *H. strigaticeps* population is the presence of retrotransposons near these sequences, which could contribute to this dispersion. This hypothesis is supported by the high concentration of *Rex*3 elements in pairs carrying 5S rDNA (pairs 25, 29, 35) in this population, which was not observed in any chromosome pairs in the population of Pirapó River. Therefore, *Rex*3 elements could be one of those responsible for this numerical variation in 5S rDNA sites between the two populations analyzed here. In *Hypostomus*, Pansonato-Alves *et al*. (2013) described an association of *Rex*1 with 5S rDNA clusters in *H. ancistroides* (Ihering, 1911) and *H. nigromaculatus*. A co-localization of *Rex*3 and rDNA 5S was also observed in the karyotype of some *Ancistrus* populations from the upper Paraná River basin (Prizon *et al*., 2018). Therefore, these data reinforce the important role of transposable elements in the chromosomal evolutionary dynamics of very diverse fish groups, such as those of the Loricariidae family.

Other fish species also evidenced TEs found in rDNA sequences or in the spacer of the rDNA sequences, such as *Erythrinus erythrinus* (Bloch & Schneider, 1801) (Cioffi *et al*., 2010), *Coregonus albula* (Linnaeus, 1758) (Symonová *et al*., 2013), *Diplodus sargus* (Linnaeus, 1758) (Merlo *et al*., 2013), *Gymnotus mamiraua* Albert & Crampton, 2001 (Silva *et al*., 2016), *Rineloricaria latirostris* (Boulenger, 1900) (Glugoski *et al*., 2018), *Megalamphodus eques* (Steindachner, 1882) (Piscor *et al*., 2020), and *Triportheus* species (Yano *et al*., 2020). This association of TEs and rDNA has also been detected in other groups of animals, plants, fungi, protozoans, and bacteria (Garcia *et al*., 2024). On the other hand, TEs are not the only agents that may contribute to rDNA spreading. Among eukaryotes, chromosomal rearrangements mediated by ectopic recombination and integration of extrachromosomal rDNA circles facilitated the spread of rDNA (Cohen, Segal, 2009; Nguyen *et al*., 2010; Proux-Wéra *et al*., 2013; Sproul *et al*., 2020; Muirhead, Presgraves, 2021; Dalíková *et al*., 2023).

Multiple 5S rDNA clusters have been observed in other *H. strigaticeps* populations (Pansonato-Alves *et al*., 2013; Baumgärtner *et al*., 2014) and other species of *Hypostomus*, such as *H. affinis* (Steindachner, 1877) (Kavalco *et al*., 2004), *H. regani* (Mendes-Neto *et al*., 2011), *H. nigromaculatus* (Pansonato-Alves *et al*., 2013), *Hypostomus* cf. *plecostomus* (Linnaeus, 1758) (Oliveira *et al*., 2015), *H. commersoni* Valenciennes, 1836 (Lorscheider *et al*., 2018), *H. regani* (Ferreira *et al*., 2019), *Hypostomus* aff. *ancistroides*, and *H. topavae* (Godoy, 1969) (Lara-Kamei *et al*., 2017), which confirms the diversity in the distribution of these sequences in *Hypostomus*.

The findings of this study have contributed to the cytogenetic characterization of *H. strigaticeps*, a species found in the upper Paraná River basin, thereby enhancing our understanding of the Loricariidae family. The study has confirmed the presence of rDNA polymorphism in the species, same in the conserved karyotype shared by the two populations. This polymorphism can be attributed to the synteny of retrotransposons with chromosomes carrying rDNA, mainly *Rex*3 elements. These transposable elements may have played a vital role in the polymorphism of this ribosomal DNA within the genome of this species.

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AUTHORS' CONTRIBUTION

Lígia Carla Balini: Conceptualization, Data curation, Investigation, Methodology, Resources, Writing-original draft, Writing-review and editing.
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Luciana Andreia Borin-Carvalho: Methodology, Project administration, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

Animals were captured with the permission of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio; number 60070–3). 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 Ethics of Animal Experiments of the Universidade Estadual de Maringá (License number: 1968010719–CEUA/UEM). The experiments followed ethical conduct, and before euthanasia, the fish were anesthetized by an overdose of clove oil (Griffiths, 2000).

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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

COMPETING INTERESTS

The authors declare no competing interests.

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