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Contributions of species *Rineloricaria pentamaculata* (Loricariidae:Loricariinae) in a karyoevolutionary context

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Abstract. Species of *Rineloricaria* demonstrate an interesting evolutionary history from a cytogenetic point of view, due to the occurrence of extensive variation in diploid number (2n=36 -70 chromosomes), with Robertsonian rearrangements mostly responsible for this karyotypic diversity. In this study we present the karyotypic data for a population of *Rineloricaria pentamaculata*, collected in the Itiz stream, a tributary of the Paraná River Basin (Paraná, Brazil), which exhibited 2n=56 chromosomes distributed in 8m/sm+48st/a (number fundamental equal to 64) and simple NOR system revealed by fluorescent *in situ* hybridization (FISH) with 18S rDNA probe, silver nitrate and positive C band, located on the first submetacentric chromosome pair (pair 5). In addition, the NOR pair showed a size heteromorphism for this region, rich in GC composition (positive CMA3). Clusters of 5S rDNA were located in 14 chromosomes and the FISH with a telomeric probe was used to map possible evidence of chromosomal fusions, however, it showed only telomeric sites. These results corroborate the data for the species *R. pentamaculata* and the genus *Rineloricaria*, showing that they are similar to most of the populations analyzed. About the cytogenetic data of *R. pentamaculata*, we reaffirm that most populations were conserved, but in those with derived characteristics, Robertsonian chromosomal rearrangements probably contributed to the karyotypic evolution of the group.

Keywords: *Rineloricaria*, chromosomal rearrangements, cytogenetics, Paraná River Basin, karyotypic evolution.

INTRODUCTION

The subfamily Loricariinae contain 252 valid species distributed by basins of Central and South America, and although morphologically the group is considered monophyletic, taxonomic problems have been reported, for example, the tribe Loricariini several species have similar descriptions (Costa-Silva 2015; Roxo et al. 2019). The genus *Rineloricaria* (Bleeker 1982) is the most numerous genus in the subfamily Loricariinae (Tribe Loricariini), currently consisting of 78 valid species (Fricke and Eschmeyer et al., 2022), distributed throughout the Neotropical region, from the Panama to Argentina, occupying a wide variety of habitats and with restricted information on genetic diversity (Rodriguez and Reis 2008; Vera-Alcaraz et al. 2012). *Rineloricaria* is also considered a monophyletic táxon, however, it presents historical taxonomic problems such as some synonymous species (*Hemiloricaria* Bleeker 1862, *Ixiandria* Regan 1906, *Fonchiichtys* Isbrücker and Michels 2001 and *Leliella* Isbrücker, 2001), species complex (*R. heteroptera* Isbrücker and Nijssen 1976, *R. lima* Kner 1853, *R. cadeae* Hensel 1868, *R. strigilata* Hensel 1868 and *R. lanceolata* Günther 1868) and doubts about the identity of the genus, due to loss of specimen data from the type locality (Costa-Silva 2015; Covain et al. 2016; Venturelli et al. 2021). *Rineloricaria pentamaculata* was described by Langeani and Araújo (1994) from specimens collected in the Turvo River (Ourinhos, SP) in the Upper Paraná River basin and has been collected in different environments of the upper Paraná River basin (Table 1).

Although cytogenetic studies in *Rineloricaria* are scarce and were performed in only 16 species (Giuliano-Caetano 1998; Alves et al. 2003; Maia et al. 2010; Rodrigues 2010; Porto et al. 2011; Rosa et al. 2012; Porto et al. 2014; Ventureli 2014, Primo et al. 2017; Guloski et al 2018, Takagui et al. 2020; Venturelli et al. 2021), these studies have contributed as an important support for studies in species taxonomically complex. Considerable karyotype diversity has been reported in genus, with diploid numbers ranging from 2n=36 in *Rineloricaria latirostris* (Giuliano-Caetano 1998) to 2n=70 in *R. lima* (Rosa et al. 2012). In addition, chromosomal polymorphisms (structural and numerical) were found in six species, called: *R. latirostris* (Giuliano-Caetano 1998), *R. pentamaculata* (Porto et al. 2011; Primo et al. 2017), *R. lima* (Rosa et al. 2012), *R. lanceolata* (Porto et al. 2014).

Robertsoninan chromosomal rearrangements are suggested as the main involved in of karyotypic evolution of *Rineloricaria*, as they would explain the origin of chromosomal polymorphisms and the extensive numerical and structural chromosomal diversity detected in species of genus (Alves et al. 2005; Porto et al. 2011; Porto et al. 2014). This hypothesis has been investigated and supported due to evidence of occurrence of these rearrangements in chromosomes of some species. Cytogenetic techniques have supported this proposition, such as FISH using 5S rDNA and telomeric probes, showed interstitial telomeric sites (ITS) co-located with 5S rDNA sites on specific chromosomes, in addition to detecting transposable elements associated with sequences of 5S rDNA. (Rosa et al. 2012; Porto et al. 2014; Primo et al. 2017; Guloski et al. 2018).

Cytogenetic studies carried out in *Rineloricaria pentamaculata* show that most populations have conserved characteristics, in relation to karyotype, location and simple NOR system and distribution of constitutive heterochromatin. However, in some populations cytogenetic diversity was observed, due to reports of B chromosomes, intrapopulational and interpopulational karyotypic differences, multiple NOR system and variation with respect to the location and amount of chromosomes with 5S rDNA sites 5S (Porto et al. 2010- 2011; Venturelli 2014; Primo et al. 2017).

This study consists of the cytogenetic characterization of *R. pentamaculata* from the Itiz stream (Rio Ivaí sub-basin, Upper Rio Paraná Basin) located in the city of Marialva (Paraná-Brazil), using classical cytogenetic techniques and physical chromosomal mapping of 18S and 5S rDNA, telomeric sequences. Thus, we compile and discuss the cytogenetic data available for the species *R. pentamaculata*, highlighting the similarities and variations found in the different populations with inferences about karyoevolutionary aspects in this group.

MATERIALS AND METHODS

Cytogenetic analysis was conducted on 16 specimens (9 females and 7 males) identified as *Rineloricaria pentamaculata* (NUP 17750 1), collected from the Itiz stream, a small tributary of the Ivaí River (basin of the upper Paraná River, Paraná state, Brazil), Voucher specimens were deposited in the Ichthyological Collection of the Limnology, Ichthyology and Aquaculture Research Center (Nupélia) at Maringá State University, Paraná, Brazil. The protocols used in this study were submitted and reviewed by the Ethics Committee on Animal Experimentation (Protocol 07/2011) of the Maringá State University.

The mitotic chromosomes of *R. pentamaculata* were obtained from kidney cells as described by Bertollo et al. (1978). Chromosomal banding was performed for detection of constitutive heterochromatin by the C-band

technique (Sumner 1972) and double staining using the fluorochromes chromomycin A3 (CMA_3) and DAPI, according to Schweizer (1976). Nucleolus organizer regions were labeled by silver nitrate ($Ag\text{-}NO_3$) staining as described by Howell and Black (1980). Fluorescent in situ hybridization (FISH) using 18S and 5S rDNA probes was performed based on Pinkel et al (1986).

The 18S rDNA probes were obtained from cloned and amplified fragments of *Prochilodus argenteus* Spix and Agassiz, 1829 (Hatanaka and Galetti 2004), the 5S rDNA probe was isolated from the genomic DNA of *Leporinus elongatus* Valenciennes, 1850 (Martins and Galetti 1999). In this study, we also used a telomeric DNA probe amplified by PCR, free of DNA, from primers (TTAGGG)n and (CCCTAA)n, based on the method of Ijdo et al. (1991). The rDNA probes were labeled by Nick translation with biotin-16-dUTP and 5S and telomeric digoxigenin-11-dUTP. Fluorescent signals were detected with avidin-FITC (for 18S rDNA) and with digoxigenin-rhodamine for 5S rDNA probes and telomeric probe. The metaphases were photographed in an Zeiss Axioskop Microscope with image capture and epifluorescence system. The morphology of the chromosomes was established according to the ratio of arms (RB), according to the proportions proposed by Levan et al. (1964), classifying them as metacentric RB from 1.00 to 1.70), submetacentric (RB from 1.71 to 3.00), subtelocentric (RB from 3.01 to 7.00) and acrocentric (RB greater than 7.00). To calculate the fundamental number (NF), metacentric (m) and submetacentric (sm) chromosomes were considered to have two arms, while subtelocentric (st) and acrocentric (a) chromosomes were single-armed.

RESULTS

The specimens of *Rineloricaria pentamaculata* had a diploid number of 2n= 56 chromosomes, with 8 m/sm+48st/a and fundamental number (NF) of 64, in both sexes (Figure 1a). Ag-NOR sites were found the entire short arm of the first pair of subtelo-acrocentric chromosomes (pair 5), showed a heteromorphism in the size of the secondary constriction (Figure 1a, in box), confirmed by FISH with an 18S rDNA probe (Figure 1b). FISH using 5S rDNA probe revealed 14 subtelo/acrocentric chromosomes with sites (pairs: 6, 7, 8, 10, 11, 12 and 14) located in the terminal regions of these chromosomes (Figure 1b). Hybridization with a telomeric probe revealed markings in the telomeric regions of all chromosomes and absence of interstitial telomeric sites (ITS) (Figure 1c).

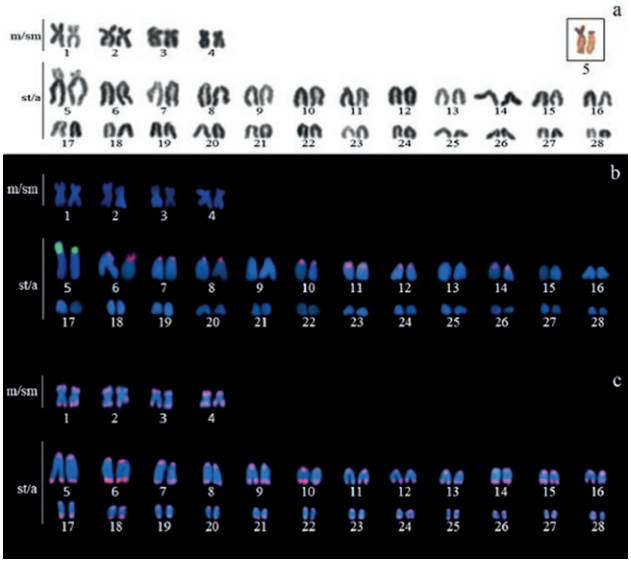

Figure 1. Karyotype for *Rineloricaria pentamaculata* of the Itiz stream after: a) conventional staining by Giemsa and Ag-NOR located on par Nº 5 (in box); b) double-FISH using 18S rDNA (green) and 5S rDNA (red) probes; **c)** FISH with telomeric probe. Note the absence of the ITS.

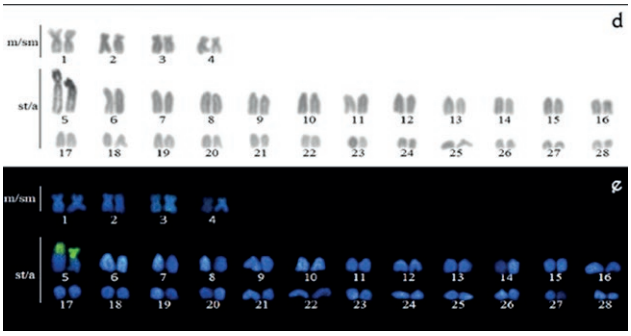

Figure 2. Karyotype of *Rineloricaria pentamaculata* from the Itiz stream showing: d) the heterochromatin distribution pattern after C-banding; **e)** CMA3/DAPI base-specific profile.

Few blocks of heterochromatin were detected in some chromosomes, however, the first pair of subtelo/acrocentric chromosomes presented blocks conspicuous associates the NOR sites (Figure 2d), which also revealed double staining with CMA_3 /DAPI and, therefore, rich in CG at the sites Ag-NOR (Figure 2e).

DISCUSSION

The data obtained in the present study showed similarities to those observed for most populations of *R.*

pentamaculata, in relation to diploid number (2n=56), karyotypic formula (8m/sm+48st/a), fundamental number (NF=64) and simple NOR system located on the first pair of st/a chromosomes (Table 1). However, divergent karyotypes were found in the populations from the Upper Paraná basin (PR), that is, 2n=58 e 2n=54 and distinct karyotypic formulas (Table 1). In the population of *R. pentamaculata* from the Barra Grande river, Primo et al. (2017) registered a karyomorph B (2n=54) and founded traces of ITS in the centromeric region of a pair of metacentrics (pair 1) suggesting the occurrence of Robertsonian fusion that resulted in the reduction of 2n from 56 to 54 chromosomes. In the population of the Tauá stream (Porto et al. 2011), two karyotypic formulas were detected and the one with 9m/sm +47st/a originated from 8m/sm + 48st/a, and that meiotic nondisjunction and chromosome fusion mechanisms promoted this karyotypic alteration, besides, B microchromosomes (0-3B) were also described for this population, whose origin has been suggested as centric fragments originated from chromosome rearrangements (Porto et al. 2010; Table 1). Therefore, we suggest that the cytogenetic characteristics detected in the present study and in most populations of *R. pentamaculata*, be considered a primitive condition for the species. In the Loricariidae family, from a cytogenetic point of view, the diploid number of 2n=54 chromosomes has been suggested as a plesiomorphic characteristic, and that due possible chromosomal rearrangements such as fusions and fissions occurred throughout the evolution of loricariids, promoted the increase and decrease of diploid numbers (Artoni and Bertollo 2001; Kavalco et al. 2005, Mendes-Neto et al. 2011; Alves et al. 2012).

Possible promoters of chromosomal rearrangements and consequently of chromosomal polymorphisms were investigated in the species of *Rineloricaria*. Interstitial telomeric sites (ITS) have been related to the centric fusion events, corroborating the hypothesis that these rearrangements the involvement with changes in karyotypic formulas, NF and reduction of diploid number in some populations of *Rineloricaria,* such as in *R. lanceolata* (Porto et al. 2014), *R. latirostris* (Primo et al. 2017) and in two species from the Iguaçu River (*R. cubatoni* and *R. maackii*, in preparation). Primo et al. (2017) conducted cytogenetic analysis on a population of *R. pentamaculata* from the Barra Grande River whose specimens showed diploid number of 2n=54 chromosome with the first metacentric chromosome pair bearer of an ITS. The telomeric sequences located in an interstitial position it has been considered traces of centric fusion occurred between acrocentric chromosomes originating metacentric chromosomese with consequent reduction of the diploid number from 56 to 54 chromosomes and alteration of karyotypic formula (table 1; Primo et al. 2017). However, even though ITS were not observed in the population of the Itiz stream and the cytogenetic data show conserved characteristics, this method is not sufficient to postulate the occurrence of centric fusion chromosomal rearrangements. On the other hand, repetitive telomere-like DNA sequences that are components of heterochromatin and located in an interstitial position could be misinterpreted as ITS, these sequences would

Table 1. Cytogenetic data available for *Rineloricaria pentamaculata.*

River/Basin/State	2n (NF)	Karyotype Formula	NOR		rDNA 5S	Ref
			Ag-NOR	rDNA 18S		
Taquaral River/ Paranapanema/ SP	58(62)	4m/sm+54st/a	te (1th st/a)	te (1th st/a pair 3)	10 sites/te	1
Juruba/ Tibagi River/ PR	56(70)	14m/sm+42st/a	-	te (1th st/a, pair 3)	12 sites/te	
Barra Grande River/ Ivaí River / PR	56(70)*	14m/sm+42st/a	-	te (1th st/a, pair 3)	10 sites/te	2
	54(64)**	10m/sm + 44st/a	-	te (1th st/a, pair 4)	8 sites/te	
Tauá Stream/ Alto Paraná River basin / PR	56(65)	9m/sm+47st/a	te (1th st/a)	te (1th st/a)	-	3
	56(64)	8m/sm+48st/a	te (1th st/a)	te (1th st/a)	-	
Tatupeba River/ Alto Paraná River basin PR	56(64)	8m/sm+48st/a	te (1th st/a)	te (1th st/a) and 4th st/a)	-	
Keller River/ Alto Paraná River basin / PR	56(64)	8m/sm+48st/a	te (1th st/a)	te (1th st/a)	-	
Jacucaca River/ Alto Paraná River basin / PR	56(64)	8m/sm+48st/a	te (1th st/a)	te (1th st/a)	12 sites	4
Água do Oito Stream/Tibagi River/ Alto Paraná River	56(64)	8m/sm+48st/a	te (1th st/a)	-	-	5
Quexada River/ Alto Paraná River / PR	56(64)	8m/sm+48st/a	te (1th st/a)	te (1th st/a)	12 sites	6
Itiz stream/ Ivaí River / Alto Paraná River PR	56(64)	8m/sm+48st/a	te (1th st/a)	te (1th st/a)	14 sites/te	7

Subtitles: 2n: diploid number; NF: fundamental number; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric; te: terminal; SP: São Paulo; PR: Paraná; Ref: references: 1- Rodrigues 2010; 2- Primo et al. 2017: * karyomorph A and ** Karyomorph B;.3- Porto et al. 2011; 4- Maia et al. 2010 and Venturelli 2014; 5-Maia et al. 2010; 6-Venturelli 2014; 7- Present study.

not be involved with centric fusion events (Meyne et al. 1990; Ocalewicz 2013; Bolzán 2017).

In addition to ITS, other repetitive DNA sequences are considered hotspot for chromosomal rearrangements, contributing to the understanding of the karyotypic diversity of the genus. In *R. lima*, 5S rDNA sites associated with TTAGGGn were observed in centromeric position in some meta-submetacentric chromosomes suggesting as susceptible sites for chromosomal breaks (Rosa et al. 2012). Glugoski et al. (2018) found transposable elements associated with 5S rDNA sites and TTAGGGn sequences in a population of *R. latirostris* (river of Pedras, Ventania-PR Brazil) and that these elements are probably also involved with chromosomal rearrangements and the karyotypic variability of the species. However, transposable elements were not observed associated with repetitive regions of the genome (5S rDNA and/or TTAGGGn) in populations of *R. latirostris* (Laranjinha river, Ventania-PR Brazil*), R. pentamaculata, R. stellata* and *R. capitonia* (Primo et al. 2018) and this presente study, such analysis was not performed. Thus, the expansion of molecular cytogenetic studies in the genus are essential for the better understanding of the types of rearrangements that caused chromosomal variability, as well as the mechanisms involved in the karyotypic evolution of *Rineloricaria*.

The simple NOR system located in the terminal position of the first pair of subtelo-acrocentric chromosomes is a conserved characteristic among the populations of the species and *Rineloricaria* genus, and was also detected in the present study (Alves et al. 2003; Rodrigues 2010; Maia et al. 2010; Porto et al. 2011; Rosa et al. 2012; Porto et al. 2014; Primo et al. 2017; Venturelli et al. 2021), except for *R. pentamaculata* from the Tatupeba stream, which showed NOR system multiple with two pairs of subtelo-acrocentric chromosomes containing 18S rDNA sites, one of the pairs being the first pair of subtelo-acrocentric chromosomes (Porto et al. 2011; Table 1). The NOR phenotype detected in the present study and in most populations of *R. pentamaculata* and in other species of *Rineloricaria* indicates an origin from a common ancestor. In the Loricariidae most species, showed that the simple NOR system located in terminal position and constitutive heterochromatin generally associated with this region are suggested as an ancestor phenotype. (Júlio-Jr 1994; Artoni and Bertollo 1996; Ribeiro et al. 2015; Prizon et al. 2016; Venturelli et al. 2021). However, in species of Loricariidae with multiple NOR system and the occurrence of B chromosomes, these characteristics were considered apomorphic. (Artoni and Bertolo 1996; Artoni and Bertolo et al. 2001; Kavalco et al. 2005; Porto et al. 2010-2011; Rubert et al. 2016).

Likewise, spécimens of *R. pentamaculata* from the Itiz stream showed similarity to other cytogenetic studies in the pattern of constitutive heterochromatin distribution (Maia et al. 2010; Porto et al. 2011; Venturelli 2014; Primo et al. 2017). The association of constitutive heterochromatin and NOR, detected in the present study, has been frequently reported in fish and shared by all species of *Rineloricaria* has been interpreted as a synapomorphic trait and comes from a common ancestor. (Giuliano-Caetano 1998; Porto et al. 2011; Venturelli et al. 2021). In addition to corroborating the data on the distribution of constitutive heterochromatin in *R. pentamaculata*, we show the composition of CG-rich heterochromatin (CMA3 positive) and emphasize the importance of descriptive and comparative cytogenetics. Furthermore, it is observed that most species of *Rineloricaria*, especially *R. pentamacula*, exhibited low constitutive heterochromatin profiles, with a varied distribution being found in the interstitial and pericentromeric regions, occupying low portions of the long or short chromosome arms (Primo et al. 2017).

Physical mapping of 5S rDNA sequences in species of *Rineloricaria,* especially in *R. pentamaculata*, has increased since 2011 and of the 11 cytogenetically characterized species, seven presented studies of 5S rDNA sites, showing variation in location and quantity (7 to 14 sites, table 1). For the genus *Rineloricari*a, these regions are evidenced in more than one pair of chromosomes (Rosa et al. 2012; Primo et al. 2017; Glugoski et al. 2018; Venturelli et al. 2021), however, it is not possible to establish a pattern, suggesting that these sites is a species-specific character. Information on diploid number and 5S rDNA distribution, has been used as a support to distinguish species of *Rineloricaria* that are morphologically similar, with difficulties in characterizing and validating the taxonomic status of the species. (Venturelli et al, 2021).

A hypothesis that could explain the multiple sites of 5S rDNA detected in species of *Rineloricaria* is that tranposable elements promoted the dispersion of copies of these genes throughout the genomes (Primo et al. 2018; Glugoski et al. 2018). Furthermore, according to Glugoski et al. (2018) in *R. latirostris*, showed multiple degenerate 5S rDNA, would be involved with the insertion of the transposable element hAT. Unequal crossover has been suggested to explain the existence of these degenerate sequences, establishing a breakpoint region susceptible to chromosome breakage, non-homologous recombination and Robertsonian fusion (Rb), and thus corroborating the hypothesis that both 5S rDNA sites and transposable elements may be involved with chromosomal polymorphisms and the karyotypic variability

observed in *Rineloricaria* (Glugoski et al. 2018).

Therefore, the present study contributes to aggregate and stimulate cytogenetic studies in the species *Rineloricaria pentamaculata* and others species of *Rineloricaria*. Futhermore, we postulate that the diploid number of 2n=56, karyotypic formula 8m/sm + 44st/a, NF=64 and simple NOR system located in the first pair of ts/a chromosomes reinforcing this chromosomal structure as representative of this species and probably, a plesiomorphic condition for *R. pentamaculata*. On the other hand, for those populations that presented apomorphic cytogenetic characteristics (Tauá and Tatupeba streams and Barra Grande river, Table 1), Robertsonian rearrangements could have caused these variations, and that 5S rDNA sequences and the transposable elements promoted these rearrangements, contributing for the karyotypic evolution to the species. However, the presence of multiple 5S rDNA sites also seems to be a characteristic of the chromosomal structure of *R. pentamaculata*, constituting an important marker of intraspecific variations in comparative analyzes of this group.

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REFERENCES

Alves ALC, Oliveira C, Foresti F. 2003. Karyotype variability in eight species of the subfamilies Loricariinae and Ancistrinae (Teleostei, Siluriformes, Loricariidae). Caryologia. 1:57–63.

Alves AL, Oliveira C, Foresti F. 2005. Comparative cytogenetic analysis of eleven species 308 of subfamilies Neoplecostominae and Hypostominae (Siluriformes: Loricariidae). Genetica. 309(124):127-136.

Alves AL, Borba RS, Oliveira C, Nirchio M, Granado A, Foresti F. 2012. Karyotypic diversity and evolutionary trends in the Neotropical catfish genus *Hypostomus* Lacépède, 1803 (Teleostei, Siluriformes, Loricariidae). Comp Cytogen. Available from: http://www.pensoft.net/journals/compcytogen.

Artoni RF, Bertollo LAC. 2001. Trends in the karyotype evolution of Loricariidae fish (Siluriformes). Hereditas. 134:201–210. Available from: http://doi/10.1111/j.1601-5223.2001.00201.x

Artoni RF, Bertollo LAC. 1996. Cytogenetic studies on Hypostominae (Pisces, 312 Siluriformes, Loricariidae). Considerations on karyotype evolution in the genus *Hypostomus*. Caryologia. 49:81–90.

Bertollo LAC, Takahashi CS, Moreira Filho O. 1978. Cytotaxonomic considerations on *Hoplias lacerda* (Pisces, Erythrinidae). Brazilian Journal of Genetics. 1: 103–120. Bolzán AD. 2017. Interstitial telomeric sequences in vertebrate chromosomes: origin, function, Instability and evolutions. Mutation Research. 1383-5742(16):30153-3. Available from: http://dx.doi.org/doi:10.1016/j.mrrev.

Costa-Silva GJ, Rodriguez MS, Roxo FF, Foresti F, Oliveira C. 2015. Using Different Methods to Access the Difficult Task of Delimiting Species in a Complex Neotropical Hyperdiverse Group. PLoS ONE. 10(9):e0135075. Available from: http://doi/10.1371/journal. pone.0135075

Covain R, Fish-Muller S, Oliveira C, Mol JH, Montoya-Burgos JI, Dray S. 2016. Molecular phylogeny of the highly diversified catfish subfamily Loricariinae (Siluriformes, Loricariidae) reveals incongruences with morphological classification. Mol Phylogenet Evol. 94:492–517. Available from: https://doi.org/10.1016/j.ympev.2015.10.018

Kavalco KF, Pazza R, Bertollo LAC, Moreira-Filho. 2005. Karyotypic diversity and 370 evolution of Loricariidae (Pisces, Siluriformes). Heredity. 4:180-186.

Fricke R, Eschmeyer WN. 2022. A guide to fish collections in Eschmeyer's catalog of fishes [internet]. San Francisco: California Academy of Science. Accessed [2022-03-02]. Available from: http://researcharchive.calacademy. org/research/ichthyology/catalog/collections.asp.

Giuliano-Caetano L**.** 1998. Polimorfismo cromossômico Robertosiano em populações de *Rineloricaria latirostris* (Pisces, Loricariidae). [thesi]. Departamento de Genética e Evolução. São Carlos-SP: Universidade Federal de São Carlos.

Glugoski L, Giuliano-Caetano L, MoreiraFilho O, Vicari MR, NogarotoV. 2018. Co-located hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. Gene. 650:49-54. Available from: http://doi/10.1016/ j.gene. 2018.01.099.

Hatanaka T, Galetti Jr PM. 2004. Mapping 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1929 (Characiformes, Prochilodontidae). Genetica. 122:239–244. DOI:10.1007/s10709-004-2039-y.

Howell WM, Black DA. 1980. Controlled silver-staining of nucleolus organizer regions with a protective

colloidal developer: a 1-step method. Experientia. 36:1014–1015. DOI:10.1007/ BF01953855.

Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA. 1991. Origin of human chromosome 2: an ancestral telomere-telomere fusion. Proc Natl AcadSci USA. 88:9051-9055.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52:201–220. Available from: DOI:10.1111/j.1601-5223.1964.tb01953.x.

Maia TPA, Giuliano – Caetano L, Rodriguez MS, Rubert M, Takagui FH, Dias AL. 2010. Chromosomal banding in three species of the genus *Rineloricaria* (Siluriformes, Loricariidae, Loricariinae).Ichthyol Res. 57:209–213.

Mendes-Neto EO, Vicari MR, Artoni RF, Moreira-Filho O. 2011. Description of karyotype in *Hypostomus regain* (Ihering, 1905) (Teleostei, Loricariidae) from the Piumhi river in Brazil with comments on karyotype variation found in Hypostomus. Comparative Cytogenetics. 5:133–142. DOI:10.3897/compcytogen.v5i2.964

Meyne J, Baker RJ, Hobart H, Hsu TC, Oliver AR, Ward OG. 1990. Distribution of non-telomeric sites of the (TTAGGG)n telomeric sequences in vertebrate chromosomes. Chromosoma. 99:3–10.

Pinkel D, Straume T, Gray JW. 1986 Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences of the United States of America. 83:2934–2938. DOI:10.1073/pnas.83.9.2934

Porto FE, Portela-Castro ALB, Martins-Santos IC. 2010. Possible origins of B chromossomes in *Rineloricaria pentamaculata* (Loricariidae, Siluriformes) from the Paraná River basin. Genetic Molecular Research. 9(3):1.654-1659. DOI: 10.4238/vol 9-3gmr859.

Porto FE, Portela-Castro ALB, Martins-Santos IC. 2011. Chromosome polymorphism in *Rineloricaria pentamaculata* (Loricariidae, Siluriformes) of the Paraná River basin. Ichthyologic Research. 58:225–231. DOI:10.1007/s10228-011-0215-5.

Porto FE, Vieira MMR, Barbosa LG, Borin-Carvalho LA, Vicari MR, Portela-Castro ALB, Martins-Santos IC. 2014. Chromosomal polymorphism in *Rineloricaria lanceolata* Günther, 1868 (Loricariidae: Loricariinae) of the Paraguay basin (Matogrosso do Sul, Brazil): evidence of fusions and their consequences in the population. Zebrafish. 11(4):318-24. DOI:10.1089/zeb.2014.0996.

Primo CC, Glugoski L, Mara C, Almeida MC, Zawadzki CH, Moreira-Filho O, Vicari MR, Nogaroto V. 2017. Mechanisms of chromosomal diversification in species of *Rineloricaria* (Actinopterygii: Siluriformes: Loricariidae). Zebrafish. 14:161-168.

Primo CC, Glugoski L, Vicari MR, Nogaroto V. 2018. Chromosome Mapping and Molecular Characterization of the Tc1/Mariner Element in *Rineloricaria* (Siluriformes: Loricariidae). Braz. Arch. Biol. Technol. DOI:10.1590/1678-4324-2018170623.

Prizon AC, Borin-Carvalho LA, Bruschi DP, Ribeiro MO, Barbosa LM, Ferreira GEB, Cius A, Zawadzki CH, Portela-Castro ALB. 2016. Cytogenetic data on *Ancistrus* sp. (Siluriformes, Loricariidae) of the Paraguay River basin (MS) sheds light on intrageneric karyotype diversification. Comp Cytogen. 10(4): 625–636. Available from: http://doi/10.3897/CompCytogen.v10i4.8532 http://compcytogen.pensoft.net.

Rodrigues RM. 2010. Estudos cromossômicos e moleculares em Loricariinae com ênfase em espécies de *Rineloricaria* (Siluriformes, Loricariidae): uma perspectiva evolutiva. 218p. [dissertation]. São Paulo (SP): Instituto de Biociências da Universidade de São Paulo.

Rodriguez M.S, Reis RE. 2008. Taxonomic Review of *Rineloricaria* (Loricariidae: Loricariinae) from the Laguna dos Patos Drainage, Southern Brazil, with the Descriptions of Two New Species and the Recognition of Two Species Groups. Copeia. 2008:333–49.

Rosa KO, Ziemniczak K, Barros AV, Nogaroto V, Almeida MC, Cestari MM, Artoni, RF, Vicari MR. 2012. Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): fusion points carrying 5S rDNA or telomere sequence vestiges .Rev Fish Biol Fisheries. DOI:10.1007/s11160-011-9250-6.

Rubert M, Rosa R, Zawadzki CH, Mariotto S, Moreira-Filho O, Giuliano-Caetano L. 2016. Chromosome Mapping of 18S Ribosomal RNA Genes in Eleven *Hypostomus* Species (Siluriformes, Loricariidae): Diversity Analysis of the Sites. Zebrafish. 13(4). DOI:10.1089/zeb.2016.1279.

Scavone MDP, Júlio HF Jr. 1994. Cytogenetic analysis and probable supernumerary chromosomes of *Loricaria prolixa* and *Loricaria* sp. Females (Loricariidae-Siluriformes) from the Parana´ river basin. Rev Ictiol. 2:41–47.

Schweizer D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromossoma. 58: 307–324. DOI: 10.1007/BF00292840.

Sumner AT. 1972. A simple technique for demonstrating centromericheterochromation. Experimental Cell Research. 75:304–306. DOI:10.1016/0014-4827(72)90558-7.

Takagui FH, Baumgartner L, Venturelli NB, Paiz LM, Viana P, Dionısio JF, Pompeo LRS, Margarido VP,

Fenocchio AS, Rosa R, Giuliano-Caetano L. 2020. Unrevealing the Karyotypic Evolution and Cytotaxonomy of Armored Catfishes (Loricariinae) with Emphasis in *Sturisoma*, *Loricariichthys*, *Loricaria*, *Proloricaria*, *Pyxiloricaria*, and *Rineloricaria*. Zebrafish. 17:5. DOI: 10.1089/zeb.2020.1893.

Venturelli NB. 2014. Mapeamentos dos genes ribossômicos e cromossomos marcadores em nove espécies de *Rineloricaria* (Siluriformes, Loricariidae, Loricariinae) de distintas Bacias hidrográficas. [dissertation]. Londrina (PR): Universidade Estadual de Londrina.

Venturelli NB, Takagui FH, Pompeo LRS, Rodriguez MS, Rosa R, Giuliano-Caetano L. 2021. Cytogenetic markers to understand chromosome diversification and conflicting taxonomic issues in *Rineloricaria* (Loricariidae: Loricariinae) from Rio Grande do Sul coastal drainages. Biologia. Available from: https://doi.org/10.1007/s11756-021-00748-3.

Vera-Alcaraz HS, Pavanelli CS, Zawadzki CH. 2012. Taxonomic revision of the *Rineloricaria* species (Siluriformes: Loricariidae) from the Paraguay River basin. Neotropical Ichthyology. 10:285–311.