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Research Article
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Chromosomal characterization of three species of Serrasalmini (Serrasalmidae: Characiformes)

Alan Gomes dos Santos[1], José Francisco de Sousa e Souza[1], Simone Cardoso Soares[1], Celeste Mutuko Nakayama[2#] and Eliana Feldberg[1,2]

[1]Instituto Nacional de Pesquisas da Amazônia (INPA), Programa de Pós-graduação em Genética, Conservação e Biologia Evolutiva, Laboratório de Genética Animal, Manaus, AM, Brazil.
[2]Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Biodiversidade, Laboratório de Genética Animal, Manaus, AM, Brazil.

Abstract

The tribe Serrasalmini is a diverse group with paraphyletic genera and taxonomic uncertainties. Several studies have been carried out in this group of fish in order to understand this problem, including the cytogenetic approach. In this study, three species of a clade of Serrasalmini were characterized cytogenetically – *Pristobrycon striolatus*, *Catoprion absconditus* and *Pygopristis denticulatus*. The three species presented diploid number (2n) equal to 62 chromosomes, of one and two arms, with karyotypic formulas and species-specific fundamental numbers. Heterochromatin is centromeric and terminal (bi-telomeric) in most chromosomes, with a conspicuous interstitial block at pair 1 (m) in all three species. The nucleolar organizer regions were multiple and C-band positive, and their location was confirmed via 18S ribosomal DNA mapping; however, with additional sites. The 5S rDNA was located in interstitial region of long arm of pair 1 (m), in the three species (homeologous). Moreover, we observed synteny between 18S and 5S in the species *C. absconditus* and *P. denticulatus*, which, according to fiber-FISH, are interspersed. Thus, the maintenance of 2n (62) evidences the diversification of chromosomal formulas within the clade by non-Robertsonian rearrangements and reflects the paraphyly of the related species.

Keywords: Piranha, chromosomal evolution, repetitive DNA, synteny.

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Introduction

Serrasalmidae constitutes a monophyletic group that has approximately 100 valid species, which are distributed in 16 genera (Fricke *et al*., 2023). They are popularly known as "pacus" and "piranhas", and are endemic to the Neotropical region, where they inhabit a wide variety of water bodies, including the main channels of rivers, lakes, flooded forests, to environments of rapids, and have wide distribution and abundance in the Amazon, Orinoco and Paraná-Paraguay basins (Goulding, 1980; Machado-Allison, 1983; Jégu, 2003; Mateussi *et al.*, 2020a).

Several studies have shown that "piranhas" and "pacus" form a well-defined group within the order Characiformes, composing the family Serrasalmidae, which is divided into three clades (Ortí *et al*., 1996, 2008; Calcagnotto *et al*., 2005; Mateussi *et al*., 2020a; Kolmann *et al*., 2021). Based on analyses of mitochondrial and nuclear sequences, Thompson *et al*. (2014) corroborated the subdivision into three clades: the "pacu clade", the "myleus clade" and the "piranha clade", the former being considered basal and the latter as more derived. Mateussi *et al*. (2020a) proposed a phylogenomic hypothesis with ultra-conserved elements, in which all living genera of the family were included, which had a new intrafamilial classification with two subfamilies: Colossomatinae Kolmann *et al*. (2021) and Serrasalminae Bleeker 1859, the latter with two tribes: Myleini Eigenmann 1903 and Serrasalmini Bleeker 1859. The morphological characteristics for each subfamily involve the absence of a pre-dorsal spine in Colossomatinae and its presence in Serrasalminae, which is continuous to the first ray of the dorsal fin in the tribe Myleini and discontinuous in Serrasalmini.

Although this division is well defined, there are significant inter- and intraspecific variations within each clade, mainly with regard to allometry and coloration patterns, which are observed during their development or reproductive stage, as well as their morphology and distribution (Nico and Taphorn, 1988; Jégu, 2003; Queiroz *et al*., 2013). Within the tribe Serrasalmini (piranhas), for example, there are taxonomic uncertainties, with divergences in the relationship between the genera *Pristobrycon* and *Serrasalmus*. Machado-Allison (1985), in a morphological analysis, observed the non-monophyly of *Pristobrycon*, with species more related to *Serrasalmus*, and only *P. striolatus* closer to the genus *Pygopristis*. According to Ortí *et al*. (1996, 2008) and Thompson *et al*. (2014), *P. striolatus* is more closely related to *Catoprion* and *Pygopristis denticulatus*, and the other species of *Pristobrycon* (e.g., *Pristobrycon calmoni*) were grouped within *Serrasalmus*. The genus *Catoprion*, since its description in 1844 was considered monotypic (Fricke *et*

Send correspondence to Eliana Feldberg. Instituto Nacional de Pesquisas da Amazônia (INPA), Programa de Pós-graduação em Genética, Conservação e Biologia Evolutiva, Laboratório de Genética Animal, Av. André Araújo, 2936, Petrópolis, 69067-375, Manaus, AM, Brazil. E-mail: feldberg@inpa.gov.br

In memorium

al., 2023), and had a new species described: *C. absconditus* Mateussi, Melo & Oliveira, 2020, which occurs in the Amazon and Essequibo basins (Mateussi *et al*., 2020b).

Cytogenetic data in the family Serrasalmidae demonstrate that the fish of this family have high karyotypic diversity (Favarato *et al*., 2021). However, the clade formed by *Catoprion*, "*Pristobrycon*" and *Pygopristis* still lacks cytogenetic analysis, because, although 2n=62 has already been suggested by Nakayama (personal communication), no chromosomal data on these species are found in the literature. Thus, the objective of this study was to cytogenetically characterize *Catoprion absconditus* Mateussi, Melo & Oliveira, 2020, *Pristobrycon striolatus* (Steindachner, 1908) and *Pygopristis denticulatus* (Cuvier, 1819), which form a clade in the tribe Serrasalmini.

Material and Methods

In the present study, three species of Serrasalmini were analyzed (Table 1). These suspensions were obtained from several collections by the researcher Dr. Celeste Nakayama (*in memoriam*), in the period from 1987 to 2009, under a permanent license (Nº 28095-3) granted by the Brazilian Institute of the Environment and Renewable Resources (IBAMA).

Chromosomal preparations were obtained from the kidney, according to the protocol of Bertollo *et al*. (1978), after mitotic induction with biological yeast (Oliveira *et al*., 1988). Colchicine 0.0125% was applied *in vivo* for 50 minutes. The detection of heterochromatin followed Sumner (1972), and the staining was according to Lui *et al*. (2012), which uses a solution of propidium iodide (0.5 µL propidium iodide in 20 µL Vectashield® antifade). For the detection of nucleolar organizer regions (NORs), we used the silver crystal precipitation technique (Ag-NORs) as described by Howell and Black (1980).

Genomic DNA extraction was performed from the muscle tissue and liver of the species under study, which was preserved in 100% ethanol, using the Wizard® extraction kit (Promega), following the manufacturer's recommendations. The repetitive sequences, used as a probe, were isolated using PCR. The isolation of the 18S and 5S ribosomal genes was performed using the following primers: 18S: 18S*f* (5'-CCG CTT TGG TGA CTC TTG AT-3') and 18S*r* (5'-CCG AGG ACC TCA CTA AAC CA-3') (Gross *et al*., 2010), and 5S*f* (5'-TAC GCC CGA TCT CGT CCG ATC3') and 5S*r* (5'- CAG GCT GGT ATG GCC GTA AGC 3') (Martins and Galetti Jr, 1999). Double-stranded PCR products were obtained in a total volume of 25 µL (~100 ng of genomic DNA; 1x buffer; 0.5 unit of Taq DNA polymerase; 0.2 mM of each dNTP – dATP, dCTP, dTTP, dGTP; 0.2 µM of each oligonucleotide primer; 2.0 mM of magnesium chloride and Milli-Q water to complete the volume). The reactions were processed in thermocycler (Eppendorf Mastercycler Gradient). The PCR program was used with the following steps: 18S rDNA: 1 min at 95 °C (for denaturation of the DNA strand); 35 cycles of 1 min at 94 °C, 1 min at 56 °C (annealing) and 90 s at 72 °C (amplification) and 5 min at 72 °C (final extension). rDNA 5S: 1 min at 94 °C (denaturation); 35 cycles of 1 min at 94 °C, 1 min at 55 °C (annealing) and 90 s at 72 °C (amplification) and 5 min at 72 °C (final extension). After amplification, the PCR products were verified and quantified. For the telomeric probes, the primers (TTAGGG)5 and (CCCTAA)5 were used according to Ijdo *et al.* (1991). The PCR products, 18S ribosomal DNA (rDNA) and the telomeric sequences were labeled using the Atto Nick Translation labeling kit (Jena Bioscience) method 550 – red and the rDNA 5S, 488 – green, following the manufacturer's instructions. The fluorescence *in situ* hybridization (FISH) technique was as per described by Pinkel *et al*. (1986) and the Fiber-FISH technique was performed according to Barros *et al*. (2011). The slides that used fluorochromes (C-banding and FISH) were analyzed under an epifluorescence photomicroscope (Olympus, BX-51) using an appropriate filter. At least 30 metaphases per individual were analyzed, and the best had their image captured using the DPController image capture system and were processed using the DPManager program. To assemble the karyotypes, we used Adobe Photoshop 7.0 (version CS6), by which mitotic metaphase chromosomes were cut, paired, measured in the DPManager program, and placed in descending order of size. The morphology and classification of the chromosomes were determined according to Levan *et al*. (1964) and, to determine the number of arms (FN), the metacentric (m), submetacentric (sm) and subtelocentric (st) chromosomes were considered as having two arms and the acrocentric (a) as having only one arm.

Results

The three species presented the diploid number (2n) equal to 62 chromosomes, and *Catoprion absconditus* has an FN=118 and KF=24m+28sm+4st+6a; *Pygopristis denticulatus* has FN=114 and KF=22m+26sm+4st+10a; *Pristobrycon striolatus* has FN=112 and KF=22m+22sm+6st+12a (Figure 1a, d, g). No heteromorphic sex-chromosome was found.

The heterochromatin (HC) of the three species is located, preferably, in the centromeric and terminal (bi-telomeric) regions of the chromosomes. However, some blocks are species-specific, especially those that are interstitial (Figure 1b, e, h).

Table 1 – Number of individuals analyzed in this study, according to species and sex.

Species	Individual		Location	Coordinates	Voucher
	Male	Female			
Catoprion absconditus	10	11	Uatumã River	1°54′56.7″ S, 59°28′25″ W	INPA-ICT 059837
Pygopristis denticulatus	2	4	Demini River	1°44'45" S, 62°93'31" W	INPA-ICT 059838
Pristobrycon striolatus	9	10	Anavilhanas	2°23'41" S, 60°55'14" W	INPA-ICT 059839

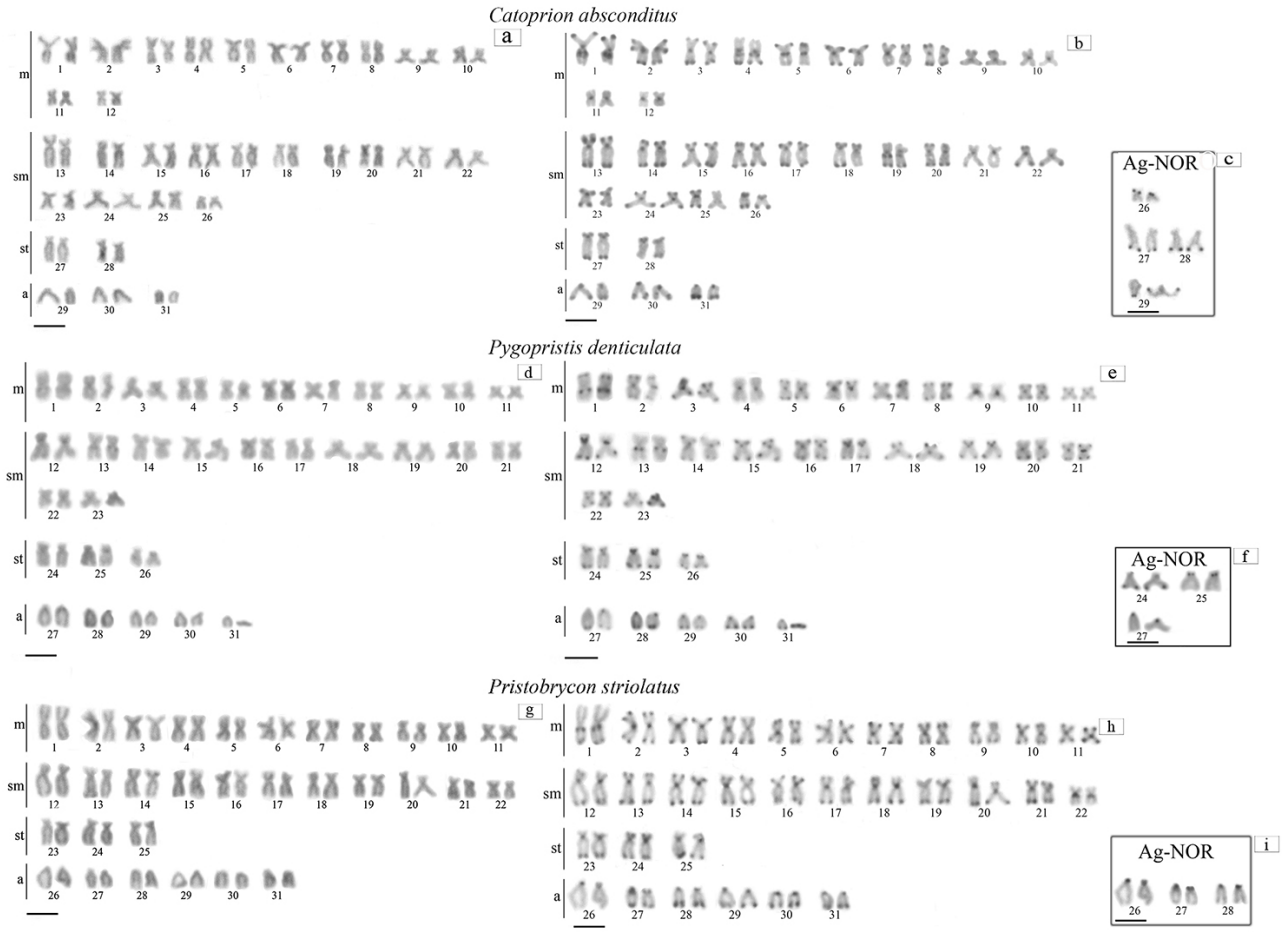

Figure 1 – Karyotypes analyzed in conventional Giemsa stain, C banding and nucleolar organizer regions (NOR, box) of *C. absconditus* (a, b, c), *P. denticulata* (d, e, f) and *P. striolatus* (g, h, i). Scale bar=5µm.

In *Catoprion absconditus*, interstitial heterochromatic markings were evidenced in the long arms of pairs 1, 4, 5 (m), 19 (sm), in the short arms of pair 13 (m), and were only centromeric in pairs 10, 11 and 12 (m) and 21 (sm) (Figure 1b). In *Pygopristis denticulatus*, interstitial markings were evidenced in the long arms of pairs 1 (m), 13 (sm) and were only centromeric in pairs 4, 9 and 11 (m). Pairs 7 and 8 (m) showed bi-telomeric markings, and pairs 6 (m) and 20 (sm) showed centromeric and telomeric markings in only one arm (Figure 1e). On the other hand, in *Pristobrycon striolatus*, interstitial blocks appeared only in pair 1 (m), and pair 22 (sm) showed only centromeric marking and pair 27 showed centromeric and terminal marking in the long arm, while the other pairs have terminal marking, which is sometimes bi-telomeric (Figure 1h).

The Ag-NORs were multiple and were evidenced in 3 to 4 chromosomal pairs. They were all C-band positive and located in the terminal portions of the chromosomes (Figure 1c, f, i). The ribosomal DNA mapping of 18S confirmed the location of the active Ag-NORs in the three species analyzed. However, additional sites were evidenced and, in *C. absconditus* and *P. denticulatus*, this additional site is in an interstitial position in pair 1 (m), colocalized with the heterochromatin block (C^+) and in synteny with 5S rDNA (Figure 2). In *P. striolatus*, we found 14 sites on acrocentric chromosomes, six coinciding with the Ag-NORs (pairs 26, 27, 28) and additional terminal markings on pairs 26*q*, 29*q*, 30*p* and 31*p*. The mapping of 5S rDNA showed only one marked pair in the three species, in an interstitial position in pair 1 (m) (Figure 2). By means of the Fiber-FISH technique, the extended fibers showed that the colocalized ribosomal genes in *C. absconditus* and *P. denticulatus* are adjacent and have the variable presence of the ribosomal DNA classes (Figure 3).

Telomeric sequences $(TTAGGG)_n$ were evidenced in the terminal portions of all chromosomes of the three species (Figure 4), and interstitial telomeric sequences (ITS) were not evidenced.

In Figure 5, we highlight the cytogenetic characteristics of the three species analyzed here, which included 2n=62, the interstitial heterochromatin block of pair 1 (m), the location of the 5S rDNA (pair 1) and the syntheny of the heterochromatic block with 5S and 18S rDNA, but not with Ag-NOR in the species *C. absconditus* and *P. denticulatus*.

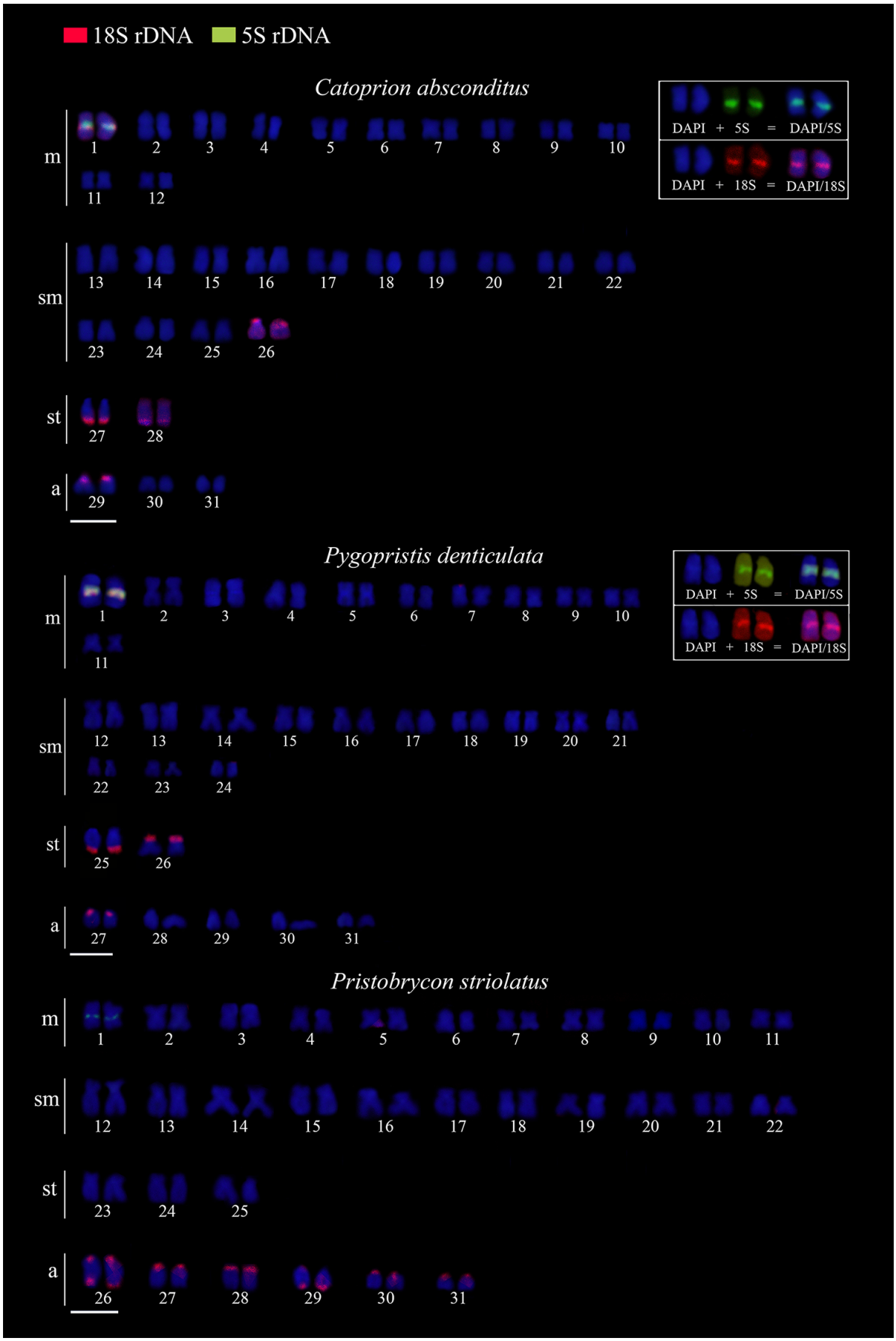

Figure 2 – Chromosomal mapping of 18S (red) and 5S (green) rDNA. Scale bar: 5µm.

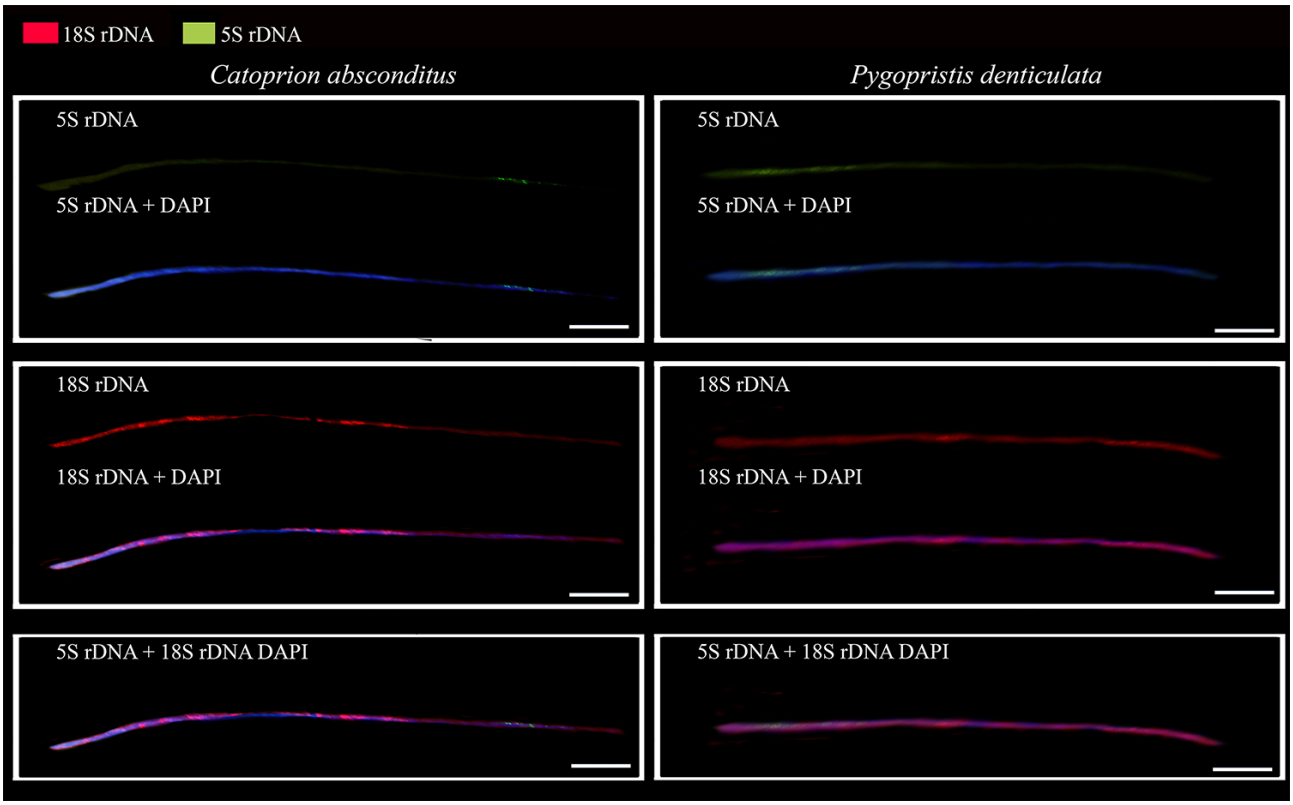

Figure 3 – Fiber-FISH with 18S (red) and 5S (green) rDNA, DAPI (blue). Scale bar: 5µm.

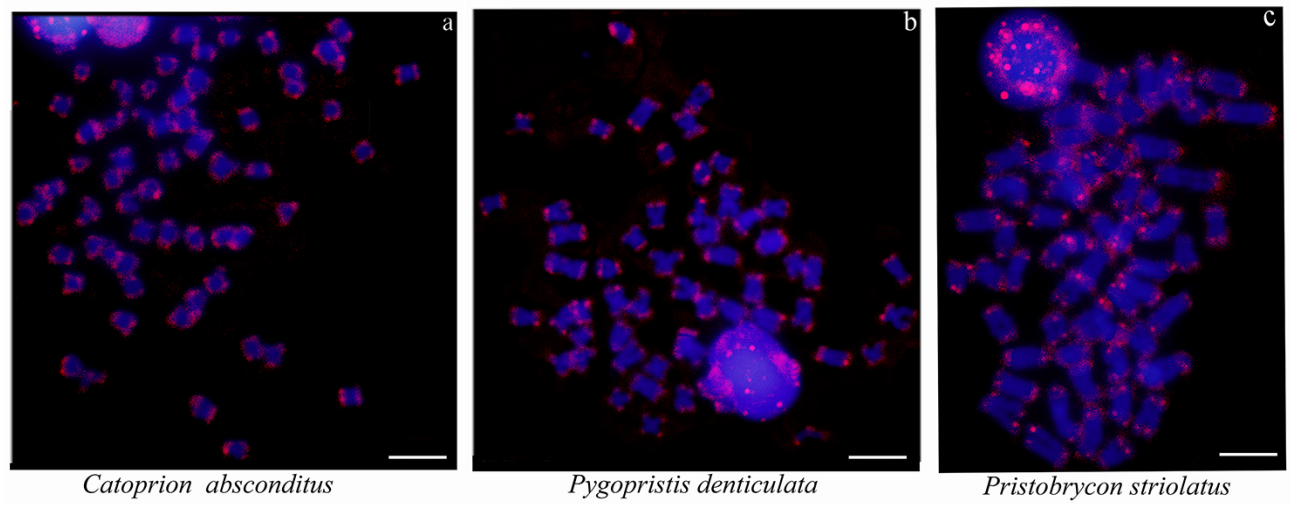

Figure 4 – Probes with telomere sequences $(TTAGGG)_n$ (red) and DAPI (blue). Scale bar: 5µm.

Discussion

In the family Serrasalmidae, the diploid number varies from 54 to 64; however, within each clade, 2n seems to be conserved (Favarato *et al*., 2021), except in *Serrasalmus* (Muramoto *et al*., 1968; Nakayama *et al*., 2002). Our data showed 2n=62 chromosomes in the three species analyzed, with 2n being shared with species of the genus *Metynnis* (Favarato *et al*., 2019, 2021). Nonetheless, although 2n is conserved among these species, the karyotypic formulas (KF) differ from each other, mainly by the number of st-a chromosomes, thus evidencing the presence of non-Robertsonian rearrangements such as inversions, translocations, duplications and heterochromatinization, given the presence of chromosomes with totally heterochromatic short arms (Figure 5).

In addition, it is curious to note the paraphyly in *Pristobrycon*, which is supported by morphological and molecular data (Machado-Allison, 1985; Mateussi *et al*., 2020a; Kolmann *et al*., 2021), which is also reflected by the cytogenetic data, since *P. striolatus* shares the diploid number with *Catoprion* and *Pygopristis* and with the only pacu of the tribe, *Metynnis*; while *Pristobrycon calmoni* (type species

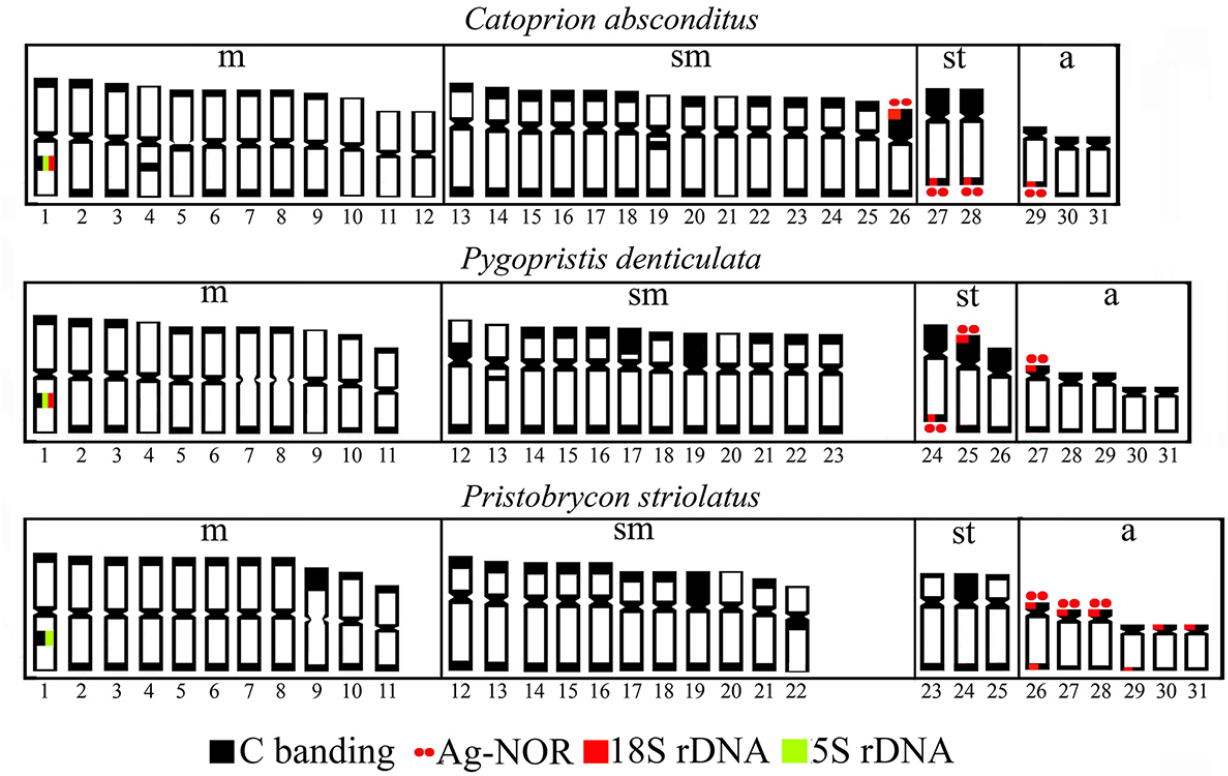

Figure 5 – Schematic representation of the chromosomes of the three species, compiling the C-band, Ag-NOR, 18S and 5S rDNA data.

of the genus) shares the 2n with the "true piranhas", such as *Serrasalmus* and *Pygocentrus* (2n=60) (Nakayama *et al*., 2008; Santana *et al*., 2011).

The location of heterochromatin, preferably in the centromeric and terminal regions (bi-telomeric), that is evidenced in the analyzed species, is common among Characiformes, and is associated with the preservation of some genes or even their silencing (Martins and Galetti Jr, 2001; Cioffi and Bertollo, 2012). In this sense, the constitutive heterochromatin blocks, here associated with the 18S and 5S rDNA sites, may be acting in the preservation of these genes or even acting in the silencing of pseudogenes associated with these sequences.

Fully heterochromatic short arms, C^+ NORs, and 18S and 5S rDNA sites coincident with C^+ blocks are common among Serrasalmidae, though species-specific interstitial blocks are also present (Nakayama *et al*., 2008, 2012; Ribeiro *et al*., 2014; Favarato *et al*., 2021). Considering the Serrasalmidae family, an increase in the diploid number and different karyotypic formulas are observed, which we associate with the presence of Robertsonian and non-Robertsonian rearrangements, but with a large participation of heterochromatin and probable mobile elements. Thus, we also observed several markers that can assist in the cytotaxonomy of the group.

A noteworthy fact is the conspicuous heterochromatic block that occurs in the interstitial position of pair 1 in the three species and is co-located with the 5S rDNA site, which has been considered a cytotaxonomic marker among Serrasalminae, since, in the genus *Serrasalmus*, this pair is in 7 (m) and in *Pygocentrus* species, this block is in pair 3 (m) (Cestari and Galetti Jr, 1992; Nakayama *et al*., 2001; Centofante *et al*., 2002 Nakayama *et al*., 2008, 2012). In this sense, we can infer that the pair carrying the interstitial heterochromatic block is homeologous among the species of the tribe Serrasalmini, and that non-Robertsonian rearrangements would have changed its position in the karyotype and, therefore, promoted diversification of the heterochromatin pattern in the different species of this tribe.

The presence of fully heterochromatic short arms in submetacentric and subtelocentric chromosomes was also observed in other representatives of the family, such as in the species *Serrasalmus* (Nakayama *et al*., 2002) and *Myloplus* (Favarato *et al*., 2021). This characteristic must have arisen due to *in tandem* duplication, especially in association with repetitive DNA elements, since the repetitive nature of these elements seems to be the trigger and target for heterochromatinization (Guimarães *et al*., 2016; Pinheiro *et al*., 2016).

The presence of chromosomal rearrangements in the evolution of the clade under study is also observed through the location of the nucleolar organizer regions (Ag-NOR and 18S rDNA), which presents wide interspecific variation. In *P. striolatus*, which has six acrocentric pairs, Ag-NOR is present in three of them, and 18S rDNA confirmed these sites; however, eight more 18S rDNA sites were detected, being a bi-telomeric pair, totaling 14 sites. In *P. denticulatus*, the Ag-NORs is present in one acrocentric pair, and in two subtelocentric pairs, with the 18S rDNA confirming the six sites of Ag-NORs but marking one more pair (1m). In *C. absconditus*, which has only three acrocentric pairs, Ag-NORs is present in one of them, and the other markings are in st and sm, with the 18S rDNA confirming these markings, but marking one more pair (1m).

The occurrence of additional 18S rDNA sites to those marked by silver nitrate, as seen in the species analyzed here, may be due to NORs dispersion events, which, due to their location in the terminal portion, are more prone to breakage, with consequent chromosomal rearrangements (Moreira-Filho *et al*., 1984; Pinheiro *et al*., 2016) or pseudogenes, by non-Robertsonian rearrangements of the translocation type or movements associated with heterochromatin or transposable elements (TEs) (Vicari *et al*., 2008; Terencio *et al.*, 2012, 2015; Guimarães *et al*., 2016).

In the genera of the basal clade of Serrasalmidae (2n=54), we found up to four sites of 18S rDNA, which, with the increase of the diploid number in the derived clades, caused the number of ribosomal sites to also increase. However, this is not true for all genera since in *Metynnis*, although its species also have 2n=62, the 18S rDNA sites are present on one or two pairs of chromosomes (Favarato *et al*., 2021). Notably, in this clade, there is an increase in the number of such sites, which reaches 14.

In the clade under study, NOR dispersal may have occurred by inversion or translocation, which are rearrangements that are closely related to ribosomal DNA families. Therefore, we suggest two scenarios: 1) pericentric inversion in nucleolar acrocentric chromosomes, which changes the morphology to two-arm chromosomes (subtelocentric/submetacentric); or 2) translocation of 18S rDNA sequences from an acrocentric to an interstitial position in metacentric chromosomes (pair 1). The first scenario is assumed due to the morphology of the chromosomes (with two arms) that maintain transcriptional activity (Ag-NOR). While in the second scenario, which did not present transcriptional activity, it would have been transposition or translocation facilitated by transposable elements (TEs), which preferentially invade rDNA regions with consequent diversity in their location within the karyotypes, due to their replicative nature (Biémont and Vieira, 2006). In *P. striolatus*, pair 26 (a) has bi-telomeric 18S rDNA and, in the region of the short arm, it is associated with HC; however, in the terminal region of the long arms, there is no HC. These sequences may be associated with TE (Terencio *et al*., 2015) and usually become inactive and prone to accumulation of mutations (insertions, deletions) at neutral rates until they completely lose their identity or become lost in the genome (Fernández-Medina *et al*., 2012).

The three species present the 5S rDNA in only one chromosome pair (pair 1), in an interstitial position, and its location in only one chromosome pair is considered an ancestral condition and may confer some advantage for protection of this gene in the genome of the species (Martins and Galetti Jr, 2000, 2001; Cioffi and Bertollo, 2012); however, several differences in number and location of this sequence (5S rDNA) were observed in the family Serrasalmidae, ranging from one to two pairs, with a terminal or interstitial position, but always colocalized with heterochromatin (Nakayama *et al*., 2008, 2012; Ribeiro *et al.*, 2014; Favarato *et al.*, 2019, 2021).

The clade that first diverged, Colossomatinae, presents the 5S rDNA sites located in two chromosomal pairs, in an interstitial position; while, in Serrasaminae, these sites are present in one or two pairs and, in species with one pair, the marking is always interstitial and, when it marks two pairs, in one, the marking is interstitial and, in the other, it is terminal (Nakayama *et al*., 2008, 2012; Ribeiro *et al*., 2014, Favarato *et al*., 2019, 2021). This indicates that non-Robertsonian rearrangements may have decreased the number of sites or that the marking in the second pair may be a pseudogene (Martins *et al*., 2002). On the other hand, we believe that the chromosomal pair that carries the 5S rDNA sequence in the interstitial position may be homeologous within the family Serrasalmidae (Nakayama *et al*., 2008, 2012; Ribeiro *et al*., 2014; Favarato *et al.*, 2019). In this sense, the great similarity between these chromosomal pairs may represent an important cytotaxonomic marker, since it allows the differentiation between species, such as those of the genera *Serrasalmus* and *Pygocentrus*, which are often confused because of their morphological similarity.

Another fact that deserves to be highlighted is the syntenic location of the 18S and 5S rDNA in *C. absconditus* and *P. denticulatus* species, for which the Fiber-FISH analysis showed the intercalated location of these rDNA sites (Figure 3). The location of the rDNA sequences on different chromosomes decreases the chances of disadvantageous rearrangements occurring during cell division (Martins and Wasko, 2004); however, in some species, the syntenic location of these rDNA sequences occur without observable damage to the host (Guimarães *et al.*, 2016; Favarato *et al.*, 2021; Souza *et al*., 2021; Moraes *et al*., 2022).

In Serrasalmidae, for example, the synteny between 18S and 5S rDNA was evidenced in three species of *Metynnis* and may confer an adaptive advantage for the maintenance of this organization (Favarato *et al*., 2021). In *Ctenolucius hujeta*, the co-location of the 18S and 5S rDNA sites was proposed as a reflection of a probable adaptive condition in the organization of these multigene families in the genome of these species (Souza *et al*., 2021). On the other hand, in Erythrinidae, in the species *Hoplias malabaricus* (Bloch, 1794), synteny may have been caused by the accumulation of repetitive DNA sequences, since the transposable elements of *Rex* 3 were mapped concomitantly with ribosomal genes (Guimarães *et al*., 2016).

The colocalization of these classes of rDNA is an unusual feature, since the dispersion of these multigene families is the result of independent events and can be associated with the silencing of these genes (Vicari *et al*., 2008; Barros *et al*., 2011; Sochorová *et al*., 2017). At this point, it is worth mentioning that, in both the species analyzed here, the 18S ribosomal DNA sites in synteny with 5S did not present transcriptional activity (using the Ag-NOR technique), which indicates that this site was inactive during the last interphase. Therefore, it is necessary to use other molecular markers, such as sequencing and characterization of gene family structures, since they could provide information about the function or pseudogenization, and cytogenetic markers, such as TE and histones, in order to better understand these events of synteny, dispersion, association and cytotaxonomic contributions (Traldi *et al.*, 2019; Haerter *et al*., 2022).

Telomeric sequences $(TTAGGG)_n$ were detected only in the terminal portions of all the chromosomes of the three species analyzed. It is interesting to note that, although the presence of rearrangements in Serrasalmidae is notorious, so far only one species analyzed presented interstitial telomeric sequences (ITS), i.e., *Colossoma macropomum* (which makes

up the basal clade of the family), in a metacentric pair, and in a centromeric position, coinciding with heterochromatin (Ribeiro *et al*., 2014).

The cytogenetic characterization of these Serrasalmini species evidenced a cytotaxonomic marker, an interstitial heterochromatin block at pair 1 (m) associated with 5S rDNA. The diversification of karyotype formulae within the clade is also observed by the nucleolar organizer regions (Ag-NOR) and 18S rDNA, which corroborates the presence of non-Robertsonian rearrangements. The 2n=62 reinforces the maintenance of the diploid number and reflects their relationship as a sister group of *Metynnis*, this being a plesiomorphic condition in the tribe. In addition, the relationship with the *Pygocentrus* + *Serrasalmus* clade indicates the occurrence of Robertsonian chromosome rearrangements that led to 2n=60. It is noteworthy that with *Pristobrycon,* considered a junior synonym of *Serrasalmus*, the results found provide further evidence (cytogenetics) that *Pristobrycon striolatus* needs a new genus in which to allocate this species.

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Conflicts of interest

The authors declare that there are no conflicts of interest that could be perceived as detrimental to the impartiality of the reported research.

Author Contributions

AGS and EF designed the study; AGS, JFSS and SCS conducted the analyses; AGS and EF analyzed the data and wrote the manuscript; AGS, JFSS, SCS and EF read and approved the final version of the manuscript.

References

Barros AV, Sczepanski TS, Cabrero J, Camacho JPM, Vicari MR and Artoni RF (2011) Fiber FISH reveals different patterns of high-resolution physical mapping for repetitive DNA in fish. Aquaculture 322-323:47-50.

Bertollo LAC, Takahashi CS and Moreira-Filho O (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erytrinidae). Braz J Genet 1:103-120.

Biémont C and Vieira C (2006) Junk DNA as an evolutionary force. Nature 443:521-524.

Calcagnotto D, DeSalle R and Schaefer SA (2005) Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. Mol Phylogenet Evol 36:135-153.

Centofante L, Porto JIR and Feldberg E (2002) Chromosomal polymorphism in *Serrasalmus spilopleura* Kner, 1858 (Characidae, Serrasalminae) from Central Amazon Basin. Caryologia 55:37-45.

Cestari MM and Galetti Jr PM (1992) Chromosome evolution in the genus *Serrasalmus* and citotaxonomic considerations about Serrasalminae (Characidae, Pisces). Braz J Genet 15:555-567.

Cioffi MB and Bertollo LAC (2012) Chromosomal distribution and evolution of repetitive DNAs in fish. In: Garrido-Ramos MA (ed) Repetitive DNA. Karger, Basel, vol. 7, pp 197-221.

Favarato RM, Ribeiro LB, Ota RP, Nakayama CM and Feldberg E (2019) Cytogenetic characterization of two *Metynnis* species (Characiformes, Serrasalmidae) reveals B chromosomes restricted to the females. Cytogenet. Genome Res 158:38-45.

Favarato RM, Ribeiro LB, Campos A, Porto JIR, Nakayama CM, Ota RP and Feldberg E (2021) Comparative cytogenetics of Serrasalmidae (Teleostei: Characiformes): The relationship between chromosomal evolution and molecular phylogenies. PLoS One 16:e0258003.

Fernández-Medina RD, Ribeiro JMC, Carareto CMA, Velasque L and Struchiner CJ (2012) Losing identity: Structural diversity of transposable elements belonging to different classes in the genome of *Anopheles gambiae*. BMC Genomics 13:272.

Goulding M (1980) The fishes and the forest: Explorations in Amazonian Natural History. University of California Press, Berkeley.

Gross MC, Schneider CH, Valente GT, Martins C and Feldberg E (2010) Variability of 18S rDNA locus among *Symphysodon* fishes: Chromosomal rearrangements. J Fish Biol 76:1117-1127.

Guimarães EMC, Carvalho NDMC, Schneider CH, Feldberg E and Gross MC (2016) Karyotypic comparison of *Hoplias malabaricus* (Bloch, 1794) (Characiformes, Erythrinidae) in Central Amazon. Zebrafish 14:80-89.

Haerter CAG, Margarido VP, Blanco DR, Traldi JB, Feldberg E and Lui RL (2022) Contributions to *Trachelyopterus* (Siluriformes: Auchenipteridae) species diagnosis by cytotaxonomic autapomorphies: From u2 snRNA chromosome polymorphism to rDNA and histone gene synteny. Org Divers Evol 1:1-18.

Howell WM and Black DA (1980) Controlled silver staining nucleolus organizer regions with protective colloidal developer: A 1-step method. Experientia 36:1014-1015.

Ijdo JW, Wells RA, Baldini A and Reeders ST (1991) Improved telomere detection using a telomere repeat probe (TTAGGG) n generated by PCR. Nucleic Acids Res 19:47-80.

Jégu M (2003) Subfamily Serrasalminae (Pacus and piranhas). In: Reis RE, Kullander SO and Ferraris Jr CJ (eds) Check list of the freshwater fishes of South and Central America. Editora da Pontifícia Universidade Católica, Porto Alegre, pp 182-196.

Kolmann MA, Hughes LC, Hernandez LP, Arcila D, Betancur R, Sabaj M, López-Fernández HH and Ortí G (2021) Phylogenomics of piranhas and pacus (Serrasalmidae) uncovers how dietary convergence and parallelism obfuscate traditinal morphological taxonomy. Syst Biol 70:576-592.

Levan A, Fredga K and Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201-220.

Lui RL, Blanco DR, Moreira-Filho O and Margarido VP (2012) Propidium iodide for making heterochromatin more evident in the C-banding technique. Biotech Histochem 87:433-438.

Machado-Allison A (1983) Estudios sobre la sistemática de la subfamília Serrasalminae (Teleostei, Characidae). Parte II. Discussion sobre la condicion monofilética de la subfamília. Acta Biol Venez 11:145-195.

Machado-Allison A (1985) Estudios sobre la subfamilia Serrasalminae. Parte III: Sobre el estatus genérico y relaciones filogenéticas

de los géneros *Pygopristis*, *Pygocentrus*, *Pristobrycon* y *Serrasalmus* (Teleostei Characidae, Serrasalminae). Acta Biol Venez 12:19-42.

Martins C and Galetti Jr PM (1999) Chromossomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiormes). Chromosome Res 7:363-367.

Martins C and Galetti Jr PM (2000) Conservative distribution of 5S rDNA loci in *Schizodon* (Pisces, Anostomidae) chromosomes. Chromosome Res 8:353-355.

Martins C and Galetti Jr PM (2001) Organization of 5S rDNA in species of the fish *Leporinus*: Two diferente genomic locations are characterized by distinct non transcribed spacers. Genome 44:903-910.

Martins C, Wasko AP, Oliveira C, Porto-Foresti F, Parise-Maltempi PP, Wrigh JM and Foresti F (2002) Dynamics of 5S rDNA in the tilapia (*Oreochromis niloticus*) genome: Repeat units, inverted sequences, pseudogenes and chromosome loci. Cytogenet. Genome Res 98:78-85.

Martins C and Wasko AP (2004) Organization and evolution of 5S ribosomal DNA in the fish genome. In: Williams CR (ed) Focus on genome research. Nova Science Publishers, New York, pp 335-363.

Mateussi NTB, Melo BF, Ota R, Roxo FF, Uchoa L, Foresti F and Oliveira C (2020a) Phylogenomics of the Neotropical fish family Serrasalmidae with a novel intrafamilial classification (Teleostei: Characiformes). Mol Phylogenet Evol 153:106945

Mateussi NTB, Melo BF and Oliveira C (2020b) Molecular delimitation and taxonomic revision of the wimple piranha *Catoprion* (Characiformes: Serrasalmidae) with the description of a new specie. J Fish Biol 97:668-685.

Moraes JN, Ferreira PV, Favarato RM, Pinheiro-Figlioulo VS and Feldberg E (2022) Karyotype variability in six Amazonian species of the family Curimatidae (Characiformes) revealed by repetitive sequence mapping. Genet Mol Biol 45:e20210125.

Moreira-Filho O, Bertollo LA and Galetti Jr PM (1984) Strtucture and variability of nucleolar organizer regions in Parodontidae fish. Can J Genet Cytol 26:564-568.

Muramoto JI, Ohno S and Atkins NB (1968) On the diploid state of the fish, order Ostariophysi. Chromosoma 24:59-66.

Nakayama CM, Jégu M, Porto JIR and Feldberg E (2001) Karyological evidence for a cryptic species of piranha within *Serrasalmus rhombeus* group (Characidae, Serrasalminae) in the Amazon. Copeia 2001:866-869.

Nakayama CM, Porto JIR and Feldberg E (2002) A comparative cytogenetic study of five piranhas species (*Serrasalmus*, Serrasalminae) from the Amazon basin. Genetica 114:231-236.

Nakayama CM, Feldberg E and Bertollo LAC (2008) Mapping of ribosomal genes and chromosomal markers in three species of the genus *Serrasalmus* (Characidae, Serrasalminae) from the Amazon basin. Genet Mol Biol 31:868-873.

Nakayama CM, Feldberg E and Bertollo LAC (2012) Karyotype differentiation and cytotaxonomic considerations in species of Serrasalmidae (Characiformes) from the Amazon basin. Neotrop Ichthyol 10:53-58.

Nico L and Taphorn D (1988) Food Habits of Piranhas in the Low Llanos of Venezuela. Biotropica 20:311-321.

Oliveira C, Almeida-Toledo LF, Foresti F, Britski HA and Toledo-Filho SA (1988) Chromosome formulae of Neotropical freshwater fishes. Braz J Genet 11:577-624.

Ortí G, Petry P, Porto JIR, Jégu M and Meyer A (1996) Patterns of nucleotide change in mitochondrial ribosomal RNA genes and the phylogeny of piranhas. J Mol Evol 42:169-182.

Ortí G, Sivasundari A, Dietz K and Jégu M (2008) Phylogeny of the Serrasalmidae (Characiformes) based on mitochondrial DNA sequences. Genet Mol Biol 31:343-351.

Pinheiro VS, Carvalho NDM, Do Carmo EJ, Schneider CH, Feldberg E and Gross MC (2016) Karyoevolution in *Potamorhina* (Cope, 1878) (Ostariophysi, Curimatidae): Using repetitive DNA for the elucidation of genome organization. Zebrafish. DOI: 10.1089/zeb.2015.1187.

Pinkel D, Straume T and Gray JW (1986) Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proc Natl Acad Sci 83:2934-2938.

Queiroz LJ, Torrente-Vilara G, Ohara WM, Pires THS, Zuanon J and Doria CRC (2013) Peixes do rio Madeira. Santo Antônio Energia Press, São Paulo.

Ribeiro LB, Matoso DA and Feldberg E (2014) Chromosome mapping of repetitive sequences in four Serrasalmidae species (Characiformes). Genet Mol Biol 37:46-53.

Santana MP, Giongo P, Travenzoli NM, Walker NJ and Sampaio WMS (2011) Diversidade cariotípica da piranha vermelha *Pygocentrus nattereri* (Characiformes: Characidae) Rio Salobo, Bacia do Araguaia, Pará, Brasil. Evol Conserv Biodiv 2:98-103.

Sochorová J, Garcia S, Galvez F, Symonova R and Kovařik A (2017) Evolutionary trends in animal ribosomal DNA loci: Introduction to a new online database. Chromosoma 127:141-150.

Souza JFS, Guimarães EMC, Pinheiro-Figlioulo VS, Cioffi MB, Bertollo LAC and Feldberg E (2021) Chromossomal analysis of *Ctenolucius hujeta* Valenciennes, 1850 (Characiformes): A new piece in the chromosomal evolution of the Ctenoluciidae. Cytogenet. Genome Res 161:195-202.

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304-306.

Terencio ML, Schneider C H, Maria C, Gross MC, Vicari MR and Feldberg E (2012) Stable karyotypes: A general rule for the fish of the family Prochilodontidae? Hydrobiologia 686:147-156.

Terencio ML, Schneider CH, Maria C, Gross MC, do Carmo EJ, Nogaroto V, de Almeida MC, Artoni RF, Vicari MR and Feldberg E (2015) Repetitive sequences: The hidden diversity of heterochromatin in prochilodontid fish. Comp Cytogenet 9:465-481.

Thompson AW, Betancur RR, López-Fernández H and Ortí G (2014) A time-calibrated, multi-locus phylogeny of piranhas and pacus (Characiformes: Serrasalmidae) and a comparison of species tree methods. Mol Phylogenet Evol 81:242- 257.

Traldi JB, Ziemniczak K, Martinez FMJ, Blanco DR, Lui RL, Schemberger MO, Nogaroto V, Moreira-Filho O and Vicari MR (2019) Chromosome Mapping of H1 and H4 Histones in Parodontidae (Actinopterygii: Characiformes): Dispersed and/or Co-Opted Transposable Elements? Cytogenet Genome Res 158:106-113.

Vicari MR, Artoni RF, Moreira-Filho O and Bertollo LA (2008) Colocalization of repetitive DNAs and silencing of major rRNA genes. A case report of the fish *Astyanax janeiroensis*. Cytogenet Genome Res 122:67-72.

Internet Resources

Fricke R, Eschmeyer WN and Van Der Laan R (2023) Eschmeyer's Catalog of Fishes: Genera, Species, References, http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed 14 March 2023).

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