

Cytogenetic analysis of *Baryancistrus xanthellus* (Siluriformes: Loricariidae: Ancistrini), an ornamental fish endemic to the Xingu River, Brazil

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Baryancistrus xanthellus is a species from the Ancistrini tribe known commonly as “amarelinho” or “golden nugget pleco”. It is one of the most popular and valued ornamental fishes due to its color pattern. Also, it is an endemic species from the Xingu River occurring from Volta Grande do Xingu, region where the Belo Monte Hydropower Dam is being built, to São Félix do Xingu. The current study aimed to cytogenetically characterize *B. xanthellus*. Results point to the maintenance of $2n=52$, which is considered the most common condition for the tribe, and a single nucleolus organizer region (NOR). Mapping of the 18S rDNA confirmed the NOR sites, and the 5S rDNA was mapped in the interstitial position of a single chromosome pair. The 18S and 5S rDNA located in different pairs constitute an apomorphy in Loricariidae. Large blocks of heterochromatin are present in pairs 1 and 10 and in the regions equivalent to NOR and the 5S rDNA. Data obtained in this study corroborated with the currently accepted phylogenetic hypothesis for the Ancistrini and demonstrate evidence that the genus *Baryancistrus* occupies a basal position in the tribe.

Baryancistrus xanthellus é uma espécie da tribo Ancistrini conhecida popularmente como “amarelinho” ou “cascudo pepita de ouro”. É um dos peixes ornamentais mais populares e valorizados, devido aos padrões de cor. Também é uma espécie endêmica do rio Xingu, ocorrendo a partir da Volta Grande do Xingu, região onde a Usina Hidrelétrica de Belo Monte está sendo construída, até São Félix do Xingu. O presente estudo teve como objetivo caracterizar citogeneticamente *B. xanthellus*. Os resultados apontam para a manutenção do $2n=52$, considerado a condição mais comum para a tribo, e região organizadora de nucléolo (RON) simples. O mapeamento do DNAr 18S confirmou a marcação da RON e o DNAr 5S foi localizado na posição intersticial de apenas um par cromossômico. A localização dos DNAr 18S e 5S em diferentes pares configura uma apomorfia em Loricariidae. Grandes blocos de heterocromatina estão presentes nos pares 1 e 10 e nas regiões equivalentes à RON e ao DNAr 5S. Os dados obtidos neste estudo corroboram a hipótese filogenética atualmente mais aceita para Ancistrini e demonstram evidências que o gênero *Baryancistrus* ocupa uma posição basal na tribo.

Keywords: FISH, Habitat endangered, rDNA, Volta Grande do Xingu.

Introduction

Loricariidae is a widespread family of fish in the Neotropical region, from Costa Rica until Argentina (Reis *et al.*, 2003). Subfamilies classification and propositions of correlations among the genera has been the subject of constant

reformulations (Reis *et al.*, 2006). According to Eschmeyer & Fong (2015) and Lujan *et al.* (2015), this family holds around 800 valid species in six subfamilies: Delturinae, Hypoptopomatinae, Hypostominae, Lithogeninae, Loricariinae, and Neoplecostominae (Schaefer, 1987; Reis *et al.*, 2003; Armbruster, 2004; Reis *et al.*, 2006).

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Ancistrini belongs to the Hypostominae and has around 217 species (Fisch-Muller, 2003) distributed in 24 genera (Armbruster, 2004; Ferraris, 2007). This tribe includes several species that are taxonomically poorly known and are often misidentified (Alves *et al.*, 2003). In addition, the majority of available studies are based only on morphological data (Isbrücker, 1980; Schaefer, 1986, 1987).

Baryancistrus Rapp Py-Daniel, 1989 is allocated into the Ancistrini and has six described species (Rapp Py-Daniel *et al.*, 2011) that are unique due to their exuberance and diversity of coloration and are, therefore, highly demanded in the fishkeeping market. The presence of yellow spots throughout its body and yellow markings on its dorsal and caudal fins characterize this species. Due to the presence of these spots, which vary in size and intensity, this species is commonly known as “amarelinho” or “golden nugget pleco”. This species is rheophilic, and its distribution is strongly linked to the rapids of the Xingu River (Rapp Py-Daniel *et al.*, 2011), which is target for several constructions to take advantage of its hydroelectric potential (Junk & Mello, 1990). Near the middle of its course, the Xingu River receives the Iri River and posteriorly suffers an accentuated deflection, forming the region known as the Volta Grande do Xingu. According to Zuanon (1999), the most commonly found species in this part of the river are from the Loricariidae family. Also, according to a report developed by several specialists on Belo Monte dam environmental impacts (Painel de Especialistas - Análise Crítica do Estudo de Impacto Ambiental do Aproveitamento Hidrelétrico de Belo Monte, 2009), the situation of the rheophilic fish there is dire.

Thus, all studies involving these species are extremely important not only for acquiring basic knowledge about

them but also to design conservation strategies, since their habitats are being seriously impacted. For the *Baryancistrus* genus, only *B. aff. niveatus* has cytogenetic data published (Souza *et al.*, 2004). Therefore, the present study investigated the conventional and molecular karyotype macrostructure of one more specie of *Baryancistrus*, *B. xanthellus*, in order to increase the information on the genetic diversity of Ancistrini on Amazon region.

Material and Methods

Thirteen specimens of *B. xanthellus* (Fig. 1) (six males, four females, and three of unidentified sex) were collected in the Xingu River in the rapids of Volta Grande do Xingu, municipality of Altamira, State of Pará (03° 36'31,5" S 51° 34'57,4" W; 03° 23'28,2" S 51° 44'29,3" W; 03° 22'29,7" S 51° 42'25,0" W; 03° 35'38,6" S 51° 49'36,0" W). Collection was performed during free dives in the rapids using a collecting permit (ICMBio SISBIO 10609-1/2007) in the name of Eliana Feldberg, and the specimens were deposited in the fish collection of INPA: INPA 43926, 43927, 43928 and 43929. The *Parecer Consubstanciado Sobre Protocolos de Pesquisas no Uso de Animais*, number 030/2013, was obtained for the experiments with the specimens.

Mitotic induction was performed with the application of a yeast solution according to the protocol of Oliveira *et al.* (1988). Mitotic chromosomes were obtained from kidney cells through the air drying technique modified for fishes by Bertollo *et al.* (1978). For the characterization of the nucleolus organizer regions (NORs), an AgNO₃ stain was done according to Howell & Black (1980). The heterochromatic regions were identified according to the protocol of Sumner (1972).



Fig. 1. Live photograph of *Baryancistrus xanthellus*, LIA 1629.

DNA extraction followed Sambrook *et al.* (1989). An amplification through PCR (polymerase chain reaction) was done for the development of 18S and 5S rDNA probes using the primers 18Sf (5'-CCG CTT TGG TGA CTC TTG AT-3') and 18Sr (5'-CCG AGG ACC TCA CTA AAC CA-3') (Gross *et al.*, 2010) and primers 5Sa (5' TAC GCC CGA TCT CGT CCG ATC-3') and 5Sb (5'-CAGGCT GGT ATG GCC GTA AGC-3') (Martins & Galetti, 1999), respectively. The final volume of each reaction was of 25µl containing 200ng of genomic DNA, 10X buffer with 1.6mM of MgCl₂, Taq DNA polymerase (5U/µl), dNTPs (1mM), primer pair (5mM) and Milli-Q water. The 18S rDNA probe obtained was isolated and labeled with digoxigenin-11-dUTP (Roche Applied Science) through the Nick Translation method and the signal detection was performed using anti-digoxigenin-rhodamine (Roche Applied Science). The 5S rDNA probe was labeled with biotin-16-dUTP (Roche Applied Science) using Nick translation and signal detection was performed using a conjugated avidin-fluorescein (FITC).

The mapping of 18S and 5S rDNA was obtained through fluorescence *in situ* hybridization (FISH), following Pinkel *et al.* (1986) with 77% stringency (2.5ng/µl of probes, formamide 50%, dextran sulphate 10% and 2xSSC [saline sodium citrate solution] at 37°C for 18h). Chromosomes were contrasted with DAPI (2 mg/mL) in a Vectashield mounting medium (Vector).

The chromosomes were analyzed in an epifluorescence Olympus BX51 microscope and the images were captured

with a mounted Olympus DP71 camera through the Image-Pro MC 6.3 software. The karyotypes were organized with the aid of the Adobe Photoshop CS6 software, measured with the ImageJ software and classified according to Levan *et al.* (1964).

Results

Baryancistrus xanthellus presents a diploid number of 52 chromosomes, (16m+28sm+8st), the fundamental number (FN) was equal 104 for males and females, and no differentiated sexual chromosomes were observed (Fig. 2). Active NOR sites were located at the interstitial portion of the short arm of the fourth metacentric pair of all the individuals analyzed. Size heteromorphism of the NOR was observed between the homologues in some specimens (Fig. 2).

Constitutive heterochromatin was found in the centromeric region in the majority of the chromosomes, extending into the proximal region of both arms in some cases. Large blocks occupied the short arms completely on pair 1 and the long arms of pair 10. The NOR was C-band positive (Fig. 3a).

Mapping of the 18S rDNA confirmed the results obtained by silver staining. As in the Ag-NOR, size heteromorphism was also observed. The 5S rDNA pattern was in the pericentromeric region of metacentric pair 7, which presented a conspicuous heterochromatic block on all analyzed specimens (Fig. 3b).

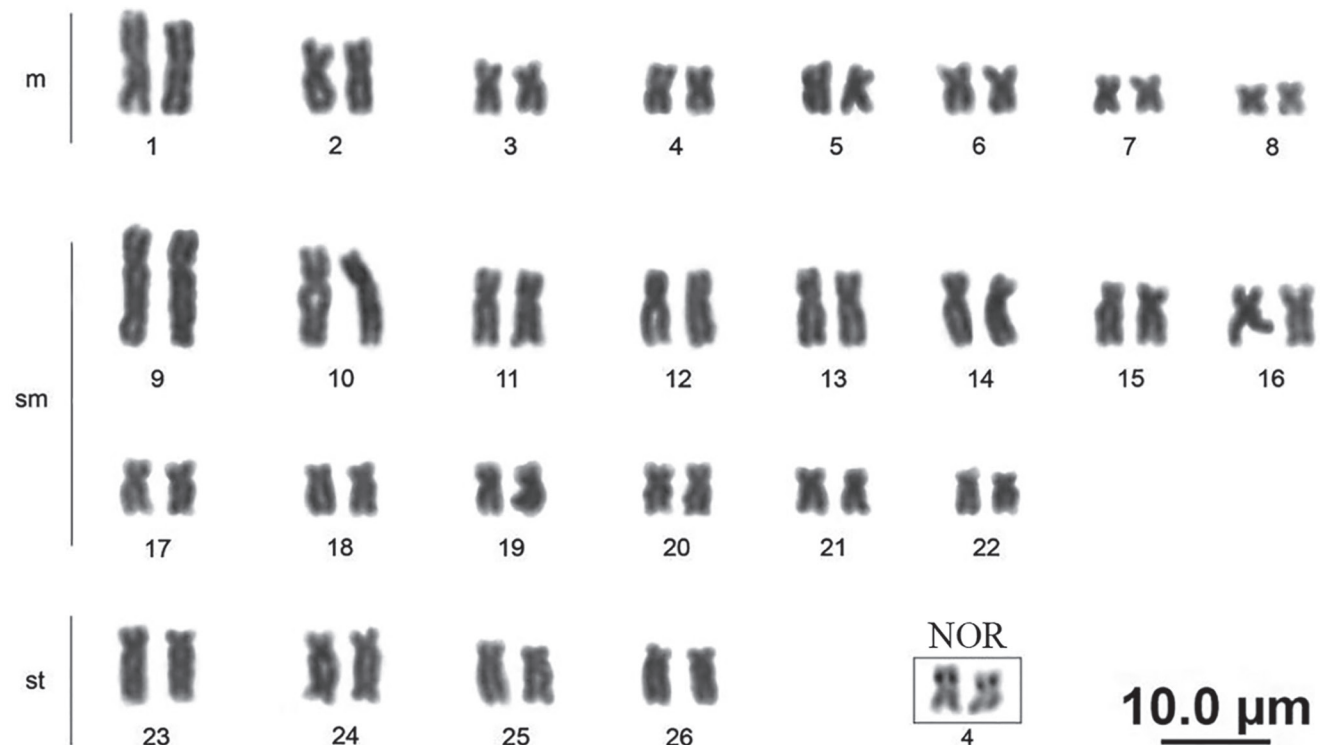


Fig. 2. Karyotype of *Baryancistrus xanthellus* in conventional staining. The square indicates the pair that bears the nucleolus organizer region (NOR).

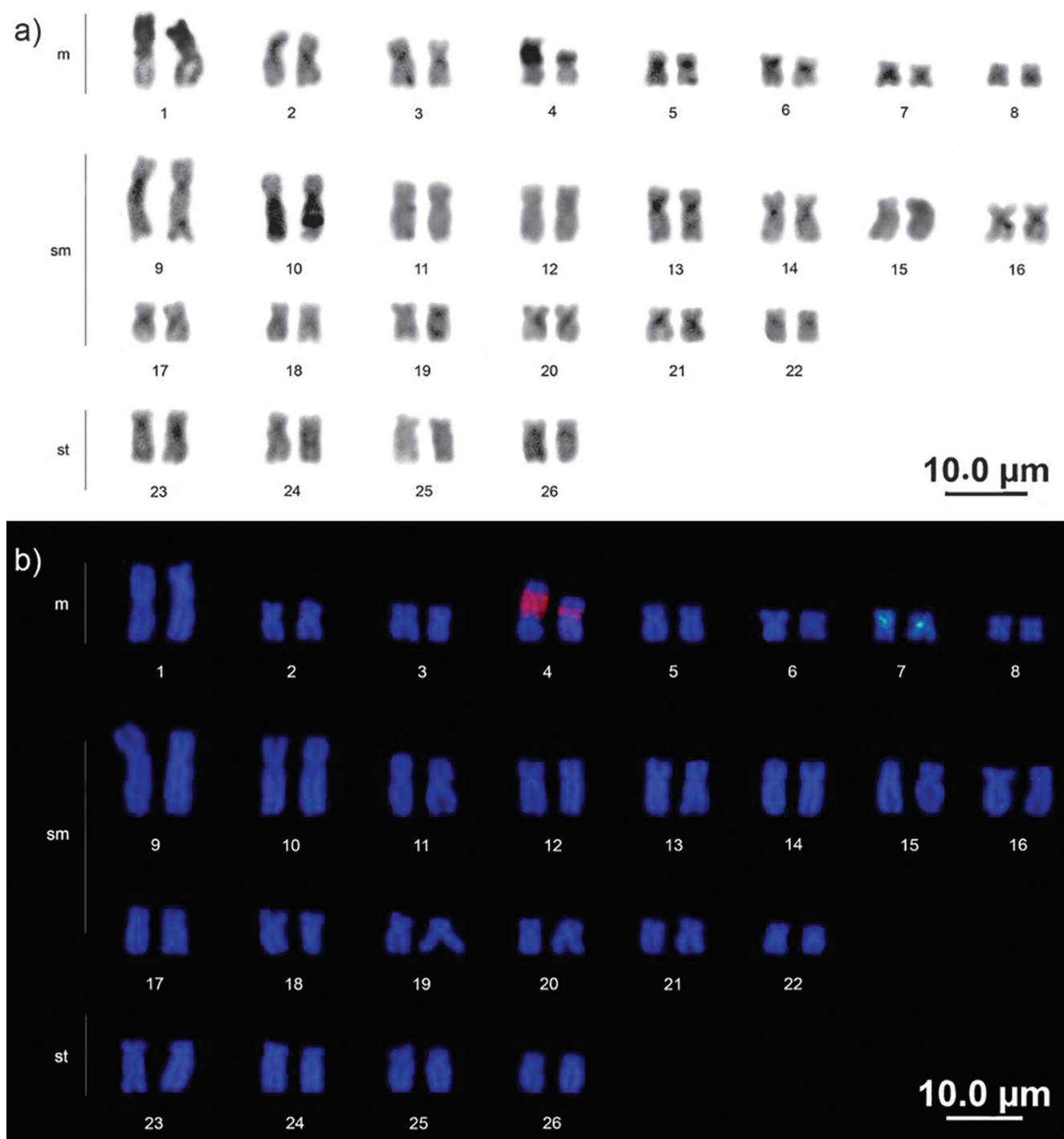


Fig. 3. Karyotype of *Baryancistrus xanthellus*: a) C-banding; b) Mapping of rDNA 18S (red signal) and 5S (green signal) through double FISH.

Discussion

Loricariidae, although with only 10% of species with any cytogenetic published data (Kavalco *et al.*, 2005), present a great karyotypic diversity in relation to the diploid number, ranging from 34 (Hypostominae) to 96 (Delturinae) (Kavalco *et al.*, 2004; Oliveira *et al.*, 2009). The diploid number of 54 chromosomes represents a

plesiomorphic condition for this group of fish (Artoni & Bertollo, 2001; Alves *et al.*, 2005; Kavalco *et al.*, 2005). To the Ancistrini, the diploid number recorded so far is ≤ 54 , indicating the presence of chromosomal rearrangements in the karyoevolution of this taxon (Oliveira *et al.*, 2009; Mariotto *et al.*, 2011). In this tribe, the most frequent diploid number is 52 chromosomes (Table 1).

Table 1. Survey of cytogenetic data of the species of Ancistrini. 2n (diploid number), FN (fundamental number), NOR (nucleolar organizer region), 18S (position that the rDNA 18S occupies in the karyotype) and 5S (pairs that have a rDNA 5S marking).

Species	Locality	2n	Karyotypic Formulae	FN	NOR	18S	5S	References
<i>Ancistrus ranunculus</i>	rio Xingu - PA	48	♂ 20m + 8sm + 6st + 14a ♀ 19m + 9sm + 6st + 14a	82	(sm) 16q proximal	(sm) 16q proximal	16	Oliveira <i>et al.</i> (2007); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp. Piagaçu	lago Aiapuí - AM	52	♂ 16m + 8sm + 2st + 26a ♀ 16m + 9sm + 2st + 25a	♂ 78 ♀ 79	(a) 26p terminal	(a) 26p terminal	1, 5, 9, 14, 15, 20, 22, 24, 25, 26	Oliveira <i>et al.</i> (2007); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp. Purus	rio Purus - AC	34	♂ 21m + 11sm + 2st ♀ 20m + 12sm + 2st	68	(m) 4p distal	(m) 4p distal	3, 5, 12, 13	Oliveira <i>et al.</i> (2009); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp. Macoari	rio Branco - RR	46	♂ 18m + 11sm + 6st + 11a ♀ 18m + 12sm + 6st + 10a	♂ 81 ♀ 82	(a) 19p distal	-	-	Oliveira <i>et al.</i> (2009)
<i>Ancistrus</i> sp. Dimona	fazenda Dimona - AM	52	16m + 8sm + 2st + 26a	78	(st) 13q distal	(st) 13q distal	♂ 1, 13; ♀ 13	Oliveira <i>et al.</i> (2009); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp. Vermelho	rio Demeni - AM	42	26m + 6sm + 4st + 6a	78	(a) 20q terminal	-	-	Oliveira <i>et al.</i> (2009)
<i>Ancistrus</i> sp. Trombetas	rio Trombetas - PA	38	22m + 8sm + 5st + 3a	73	(m) 5 centromeric	-	-	Oliveira <i>et al.</i> (2009)
<i>Ancistrus</i> sp. <i>Balbina</i>	igarapé Barretinho - AM	♂ 39 ♀ 38	♂ 27m + 10sm + 2st ♀ 26m + 10sm + 2st	♂ 78 ♀ 76	(m) 12q terminal	(m) 12q terminal	4	Oliveira <i>et al.</i> (2008); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp. <i>Barcelos</i>	rio Demeni - AM	52	♂ 12m + 12sm + 4st + 24a ♀ 11m + 12sm + 4st + 25a	♂ 80 ♀ 79	(a) 23p terminal	(a) 23p terminal	1, 2, 8, 9, 15, 16, 18, 19, 20, 22, 23, 24, 26	Oliveira <i>et al.</i> (2008); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp. <i>Catalão</i>	lago Catalão - AM	34	22m + 8sm + 4st	68	(m) 4p distal	(m) 4p distal	3, 6, 7, 12	Oliveira (2006); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp. <i>Rio Branco</i>	igarapé Macoari - RR	46	♂ 18m + 11sm + 6st + 11a ♀ 18m + 12sm + 6st + 10a	♂ 81 ♀ 82	(a) 19p distal	(a) 19p distal	19	Oliveira (2006); Favarato <i>et al.</i> (2016)
<i>Ancistrus</i> sp.	rio Iguaçu - PR	48	18m + 14sm + 12st + 4a	84	(st) p terminal	-	-	Lara (1998)
<i>Ancistrus</i> sp. 1	igarapé São Francisco - AC	38	30m/sm + 8st	76	(m/sm) 5p interstitial	-	-	Alves <i>et al.</i> (2003)
<i>Ancistrus</i> sp. 2	rio Betari - SP	52	32m/sm + 20st/a	-	(st/a) 24p terminal	-	-	Alves <i>et al.</i> (2003)
<i>Ancistrus multispinnis</i>	rio Itapocu - SC	52	28m/sm + 24st/a	-	(st/a) 17p terminal	-	-	Alves <i>et al.</i> (2003)
<i>Ancistrus</i> sp. 1	rio Vermelho - GO	♂ 39 ♀ 40	♂ 33m + 6sm ♀ 34m + 6sm	♂ 78 ♀ 80	(sm) 20q distal	-	-	Alves <i>et al.</i> (2006)
<i>Ancistrus</i> sp. 2	rio Guaruva - SC	52	10m + 16sm + 12st + 14a	90	(st) 15p distal	-	-	Alves <i>et al.</i> (2006)
<i>Ancistrus</i> sp.	rio Alto Alegre - PR	50	12m + 14sm + 14st + 10a	90	-	-	-	Tchaicka & Margarido (1999)
<i>Ancistrus cf. dubius</i>	rio Coxipó; córrego Pari; córrego Flechas; Córrego Fundo - MT	42	24m + 10sm + 8st	84	(sm) 16p interstitial	(sm) 16p interstitial	4, 14, 16	Mariotto & Miyazawa (2006); Mariotto <i>et al.</i> (2011)
<i>Ancistrus claro</i>	rio Coxipó - MT	54	14m + 8sm + 8st + 24a	84	(a) 21q interstitial	(a) 21q interstitial	4, 19, 21	Mariotto <i>et al.</i> (2011)
<i>Ancistrus citiaba</i>	baía Arrombado - MT	34	20m + 8sm + 6st	68	(m) 2p terminal	(m) 2p terminal	3, 6, 9	Mariotto <i>et al.</i> (2009); (2011)
<i>Ancistrus tombador</i>	rio Preto - MT	50	14m + 12sm + 8st + 16a	84	(a) 20p terminal	-	-	Mariotto <i>et al.</i> (2013)
<i>Ancistrus</i> sp. 01	córrego Pipa - MT	54	14m + 8sm + 8st + 24a	84	(a) 21q interstitial	-	-	Mariotto <i>et al.</i> (2013)
<i>Ancistrus</i> sp. 03	córrego Pari - MT	54	14m + 8sm + 8st + 24a	84	(a) 21q interstitial	-	-	Mariotto <i>et al.</i> (2013)
<i>Ancistrus</i> sp. 04	rio Sepotuba - MT	52	16m + 8sm + 6st + 22a	82	(a) 22q proximal	(a) 22q proximal	17, 25, 26	Mariotto <i>et al.</i> (2011)

Table 1.

Species	Locality	2n	Karyotypic Formulae	FN	NOR	18S	SS	References
<i>Ancistrus</i> sp. 06	rio Matrinxã - MT	50	18m + 10sm + 8st + 14a	86	(sm) 13p interstitial	(sm) 13p interstitial	1, 13	Mariotto <i>et al.</i> (2011)
<i>Ancistrus</i> sp. 08	rio Curupira - MT	44	18m + 10sm + 8st + 8a	80	(a) 21q proximal	(a) 21q proximal	21	Mariotto <i>et al.</i> (2011)
<i>Ancistrus</i> sp. 13	córrego Salgadoinho - MT	40	26m + 10sm + 4st	80	(sm) 18q terminal	(sm) 18q terminal	5, 15	Mariotto <i>et al.</i> (2011)
<i>Baryancistrus</i> aff. <i>Niveatus</i>	rio Xingu - PA	52	16m + 32sm + 4st	104	(m) 3p interstitial	-	-	Souza <i>et al.</i> (2004)
<i>Baryancistrus</i> sp. 1	rio Jarí - PA	52	8m + 34sm + 10st	104	(sm) 18p interstitial	-	-	Souza (2003)
<i>Baryancistrus</i> sp. 2	rio Xingu - PA	52	18m + 30sm + 4st	104	(m) 6p interstitial	-	-	Souza (2003)
<i>Baryancistrus</i> sp. 3	rio Xingu - PA	52	14m + 26sm + 12st	104	(m) 4p interstitial	-	-	Souza (2003)
<i>Hemiancistrus</i> sp.	rio Araguaia - MT	52	20m + 20sm + 12st/a	-	(sm) q terminal	-	-	Artori & Bertollo (2001)
<i>Hemiancistrus spilomma</i>	rio Araguaia - MT	52	24m + 22sm + 6st	104	(m) 2q terminal 3p terminal/(sm 18q terminal)	-	-	Oliveira <i>et al.</i> (2006)
<i>Hemiancistrus spinosissimus</i>	rio Araguaia - MT	52	26m + 22sm + 4st	104	(sm) 17q terminal	-	-	Oliveira <i>et al.</i> (2006)
					1: (m) 2q terminal	1: (m) 2q terminal	1, 9	
					2: (m) 2q terminal	2: (m) 2q terminal	1, 9	
					3: (m) 2q terminal; (st) 23q terminal	3: (m) 2q terminal (st) 23q terminal	1, 9	
<i>Hypancistrus cf. debilitera</i>	rio Uatumã - AM	52	34m/sm + 18st	104	4: (m) 2q terminal/ interstitial (1 homologous)	4: (m) 2q terminal/ interstitial (1 homologous)	1, 9	Silva <i>et al.</i> (2014)
					5: (m) 2q terminal/ interstitia (2 homologous)	5: (m) 2q terminal/ interstitial (2 homologous)	1, 9	
<i>Hypancistrus zebra</i>	rio Xingu - PA	52	38m/sm + 14st	104	(m/sm) 13q terminal	(m/sm) 13q terminal	1, 4	Silva <i>et al.</i> (2014)
<i>Lastancistrus cf. schomburgkii</i>	rio Massangana - MT	54	26m + 16sm + 12st	108	(st) 25p terminal	-	-	Mariotto (2009)
<i>Lastancistrus</i> sp.	rio Cachoetra - MT	54	26m + 18sm + 10st	108	(st) 26p terminal	-	-	Mariotto (2009)
<i>Megalancistrus oculatus</i>	rio Paraná - PR	52	26m + 26sm	104	(sm) p interstitial	-	-	Lara (1998)
<i>Panaque cf. nigrolineatus</i>	rio Araguaia - MT	52	26m + 20sm + 6st/a	-	(a) 26p terminal	-	-	Artori & Bertollo (2001)
<i>Parancistrus</i> sp. 1	rio Xingu - PA	52	20m + 26sm + 6st	104	(m) 8p interstitial	-	-	Souza (2003)
<i>Peckolia</i> sp. 1	rio Jarí - PA	52	18m + 26sm + 6st + 2a (+1B)	102	(m) 10q distal (st) 25q distal	-	-	Souza <i>et al.</i> (2009)
<i>Peckolia</i> sp. 2	rio Jarí - PA	52	16m + 16sm + 8st + 2a	102	(st) 17q distal (st) 18q distal	-	-	Souza <i>et al.</i> (2009)
<i>Peckolia vitata</i>	rio Xingu - PA	52	16m + 20sm + 14st + 2a	102	(sm) 9q distal	-	-	Souza <i>et al.</i> (2009)
<i>Scobinancistrus aureatus</i>	rio Xingu - PA	52	22m + 20sm + 10st	104	(m) 3q interstitial	(m) 3q interstitial	-	Cardoso <i>et al.</i> (2013)
<i>Scobinancistrus parolispos</i>	rio Xingu - PA	52	24m + 18sm + 10st	104	(m) 3q distal	(m) 3q distal	-	Cardoso <i>et al.</i> (2013)

With the exception of *Ancistrus*, for which the karyotypic differentiation is usually associated to a reduction in the diploid number (Oliveira *et al.*, 2007, 2008, 2009; Mariotto *et al.*, 2009), there is a tendency towards maintaining the diploid number in the tribe. However, a large amount of variation can be found in the karyotypic formulae promoted specially by pericentric inversions (Artoni & Bertollo, 2001; Alves *et al.*, 2003, 2006; Bueno *et al.*, 2012; Oliveira *et al.*, 2007, 2008; Mariotto *et al.*, 2009; Souza *et al.*, 2004, 2009; Ziemniczak *et al.*, 2012). Furthermore, chromosomal rearrangements like fusions, inversions, deletions, duplications and heterochromatinization may contribute in the differentiation of the group and may be associated to morphological speciation processes (Artoni & Bertollo, 2001; Milhomem *et al.*, 2010; Mariotto *et al.*, 2011; Ziemniczak, 2011; Bueno *et al.*, 2012). According to Ziemniczak (2011), this great karyotypic diversity might have had an important role in the genetic and reproductive isolation of the Ancistrini species.

Baryancistrus xanthellus presented $2n=52$, which was already verified in species of the genera *Peckoltia*, *Hemiancistrus*, *Hypancistrus*, *Scobinancistrus* and *Panaque* (Artoni & Bertollo, 2001; Oliveira *et al.*, 2006; Souza *et al.*, 2009; Cardoso *et al.*, 2013; Silva *et al.*, 2014). There was a single NOR located in the interstitial region of the short arm, in the third pair in *B. aff. niveatus* (Souza *et al.*, 2004) and the fourth pair in *B. xanthellus*. This character cannot be considered specific for each genus, since species from the same genus may present sites in different locations. In *Ancistrus* and *Hemiancistrus*, for example, the single sites can be found in the short or long arms, and many times even multiple NORs were found (Oliveira *et al.*, 2006, 2009). Like in *B. aff. niveatus*, the NOR was C-band positive differently from species of the genera *Peckoltia* and *Scobinancistrus* that have heterochromatic blocks adjacent to the NOR (Souza *et al.*, 2004; Souza *et al.*, 2009; Cardoso *et al.*, 2013).

In some specimens of *B. xanthellus*, the NOR was heteromorphic in regards to size when compared the homologues, which was also observed by Souza *et al.* (2004) in some specimens of *B. aff. niveatus*. This heteromorphism is very frequent in Neotropical fish and has been explained as a duplication of ribosomal genes or by a process of accumulation of these genes in one of the homologues through unequal crossing-over (Foresti *et al.*, 1981; Almeida-Toledo *et al.*, 2000; Swarça *et al.*, 2001). This might be due to the presence of constitutive heterochromatin between the ribosomal genes, which might have promoted unequal exchanges between the chromatids (Sola *et al.*, 1988) or by accumulating constitutive heterochromatin in an adjacent position to the NOR (Vicari *et al.*, 2008).

In *B. xanthellus*, large blocks of heterochromatin were observed in the short arm of pair 1 and the long arm of pair 10, and conspicuous blocks were co-located with the 18S and 5S rDNA sites (pairs 4 and 7, respectively) of all specimens

(Fig. 3a). A similar pattern of C-banding was observed in *B. aff. niveatus* where pairs 1 and 10 also presented one of the arms almost completely heterochromatic (Souza *et al.*, 2004), which could be a pattern for the genus. However, *B. aff. niveatus* also presented large blocks on pairs 11 and 22.

Ziemniczak *et al.* (2012) suggested that the absence of large and numerous blocks of heterochromatin seem to be a plesiomorphic character in Loricariidae. This characteristic may be proven by comparing the basal genera with the derived ones (Artoni & Bertollo, 2001; Oliveira, 2006; Oliveira *et al.*, 2008; Mariotto *et al.*, 2009; Traldi *et al.*, 2012). Large heterochromatic blocks are found in two or more chromosomal pairs in the genera *Scobinancistrus*, *Hypancistrus* and mainly in *Peckoltia* (Souza *et al.*, 2009; Cardoso *et al.*, 2013; Silva *et al.*, 2014). This characteristic seems to be common to the Ancistrini and corroborates the suggestion proposed by Ziemniczak *et al.* (2012).

In Siluriformes, the mapping of the ribosomal genes 18S and 5S is still very incipient (Kavalco *et al.*, 2004; Centofante *et al.*, 2006; Mendes-Neto *et al.*, 2011). In Loricariidae, chromosomes with syntenic markings of the 18S and 5S rDNA were observed in species of Neoplecostominae, Hypoptopomatinae (Ziemniczak *et al.*, 2012), Loricariinae (Kavalco *et al.*, 2004), Hypostominae (Ancistrini and Hypostomini) (Mariotto *et al.*, 2011; Traldi *et al.*, 2013) and in the outgroup, Trichomycteridae family (Ziemniczak, 2011). Based on this data, Ziemniczak (2011) inferred that the synteny of these classes of rDNA is a plesiomorphic character in the family.

For the *Baryancistrus*, this is the first record of the mapping of the rDNA 5S and 18S. The simultaneous hybridization of both probes (double-FISH) did not result in syntenic markings (Fig. 3b). This rDNA distribution in different chromosomal pairs constitutes an apomorphy in the Loricariidae (Ziemniczak, 2011).

In the Ancistrini so far, only two genera have data on 5S rDNA: *Ancistrus* (Mariotto *et al.*, 2011; Favarato *et al.*, 2016) and *Hypancistrus* (Silva *et al.*, 2014). As it has been observed for the 18S rDNA, the 5S rDNA also presented variable forms among the different species of Ancistrini. In a study by Mariotto *et al.* (2011), in which the 18S and 5S rDNA probes were hybridized in seven species of *Ancistrus*, all species presented 18S rDNA markings in a single pair. However, only one species, *Ancistrus* sp. 06, presented a single pair with 5S. This same species also presented synteny between the two ribosomal genes. The remaining species presented two or three pairs with the 5S rDNA. In regards to the position in the chromosome, 5S rDNA was found to be variable, occupying pericentromeric, interstitial, or terminal positions (Table 1).

The existence of multiple sites of the 5S rDNA in several species may be considered an important indication of the great karyotypic diversity present in Ancistrini and should correspond to an apomorphic condition in the group. Studies conducted so far in the group suggest that the localization of the 5S rDNA in a single chromosome

pair is less frequent (Table 1), occurring in a few species of *Ancistrus* (Mariotto *et al.*, 2011; Favarato *et al.*, 2016) and in *B. xanthellus*. According to Martins & Galetti (1999), the localization of the ribosomal genes in different chromosomes may be advantageous if compared to the syntenic disposition because it might avoid unfavorable arrangements (Dover, 1986), since the occurrence of unequal crossing-over might be frequent in chromosomes with co-located ribosomal genes.

In *B. xanthellus*, it is possible to visualize the heterochromatin association with 18S and 5S rDNA. This is a recurrent characteristic in the evolutionary history of Neotropical fishes (Vicari *et al.*, 2003). In some cases, the heterochromatic blocks might be adjacent to the nucleolar regions, while in other cases the markings may be overlapping or intercalated (Pendás *et al.*, 1993a,b; Artoni & Bertollo, 2001). The presence of heterochromatin, which holds large quantities of satellite DNA and transposable elements (Dimitri *et al.*, 2009), might facilitate transposition events, moving ribosomal genes to other regions of the genome (Moreira-Filho *et al.*, 1984; Vicari *et al.*, 2008; Gross *et al.*, 2009, 2010), promoting genetic duplication and unequal crossing-over, and still lead to size variation of the heterochromatin segments as well as in the number of rDNA cistrons (Sola *et al.*, 1988; Vicari *et al.*, 2003, 2008).

In general, *B. xanthellus* conserves the karyotypic macrostructure of the Ancistrini. The maintenance of $2n=52$ with a few heterochromatic blocks, a single NOR, and single rDNA sites are evidences that the genus occupies a basal position in the tribe. Our results can help to better understand the chromosomal evolution in this remarkable fish group, but the continuity of cytogenetic studies for the *Baryancistrus* is indispensable for a better comprehension of the evolutionary trends.

A karyotypic diversity might result in great morphological diversity and color pattern in the species of Ancistrini endemic to the Xingu River. This species diversity represents an invaluable richness; therefore, it is important that there are efforts to understand the origin, evolution, behavior, ecology, and the subsequent preservation of such diversity, since many species are threatened by extinction due to the changes in their original habitats caused by the construction of hydropower dams.

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