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Comparative cytogenetic analyses in *Ancistrus* species (Siluriformes: Loricariidae)

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Ancistrus is a specious genus of armored catfishes that has been extensively used for cytogenetic studies in the last 17 years. A comparison of the extensive karyotypic plasticity within this genus is presented with new cytogenetic analysis for *Ancistrus* cf. *multispinis* and *Ancistrus aguaboensis*. This study aims to improve our understanding of chromosomal evolution associated with changes in the diploid number (2n) and the dispersion of ribosomal DNAs (rDNAs) within *Ancistrus*. *Ancistrus* cf. *multispinis* and *A. aguaboensis* exhibit 2n of 52 and 50 chromosomes, respectively. Given that *A.* cf. *multispinis* shares a 2n = 52 also found in Pterygoplichthyini, the sister group for Ancistrini, a Robertsonian (Rb) fusion event is proposed for the 2n reduction in *A. aguaboensis*. 5S rDNAs pseudogenes sites have already been associated with Rb fusion in *Ancistrus* and our analysis suggests that the 2n reduction in *A. aguaboensis* was triggered by double strand breaks (DSBs) and chromosomal rearrangements at 5S rDNA sites. The presence of evolutionary breakpoint regions (EBRs) into rDNA cluster is proposed to explain part of the Rb fusion in *Ancistrus*. Cytogenetic data presented extends the diversity already documented in *Ancistrus* to further understand the role of chromosomal rearrangements in the diversification of Ancistrini.

Keywords: Armored catfish, FISH, 5S rDNA, 18S rDNA, telomeric sequence.

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Ancistrus é um gênero rico em espécies de peixes conhecidos como cascudos e tem sido alvo de estudos citogenéticos nos últimos 17 anos. Uma comparação da plasticidade presente no gênero é apresentada com novas análises citogenéticas para *Ancistrus* cf. *multispinis* e *Ancistrus aguaboensis*. Este estudo visa melhorar nossa compreensão da evolução cromossômica associada as alterações do número diploide (2n) e a dispersão de DNAs ribossômicos (rDNAs) em *Ancistrus*. *Ancistrus* cf. *multispinis* e *A. aguaboensis* apresentaram 2n de 52 e 50 cromossomos, respectivamente. Visto que *A.* cf. *multispinis* compartilha 2n = 52 também encontrado em Pterygoplichthyini, o grupo irmão para Ancistrini, um evento de fusão Robertsoniana (Rb) é proposto para a redução do 2n em *A. aguaboensis*. Sítios de pseudogenes de rDNA 5S já foram associados a eventos de fusão Rb em *Ancistrus* e nossas análises sugerem que a redução do 2n em *A. aguaboensis* foi desencadeada por quebras na dupla fita e rearranjos cromossômicos em sítios de rDNA 5S. A presença de *evolutionary breakpoint regions* (EBRs) em clusters de rDNA foi proposta para explicar parte da fusão Rb em *Ancistrus*. Os dados citogenéticos apresentados ampliam a diversidade já documentada em *Ancistrus* visando melhor entender o papel dos rearranjos cromossômicos na diversificação de Ancistrini.

Palavras-chave: Cascudo, FISH, rDNA 5S, rDNA 18S, sequência telomérica.

INTRODUCTION

Loricariidae is the largest family of the Siluriformes, which includes about 1.000 species distributed in the Neotropical region, and comprises fishes vulgarly called as armored catfishes (Fricke *et al*., 2020). It consists of six subfamilies: Delturinae, Hypoptopomatinae, Hypostominae, Lithogeninae, Loricariinae and Rhinelepinae (Armbruster, 2004; Reis *et al*., 2006). The former subfamily Ancistrinae was considered synonymous with Hypostominae by Armbruster (2004) and, currently, Hypostominae presents 483 valid species (Fricke *et al*., 2020), grouped in the tribes: Corymbophanini, Rhinelepini, Hypostomini, Pterygoplichthyini and Ancistrini (Armbruster, 2004; Lujan *et al*., 2015). In a systematic review study, Ancistrini was proposed to possess ten genera considered valid (Lujan *et al*., 2015). Previously, this tribe was distributed in a larger number of genera which ones were found to be paraphyletic, and is therefore restricted to a weakly supported clade (Lujan *et al*., 2015). Currently, Ancistrini remains a clade rich in genera and with a high morphological diversity (Lujan *et al*., 2015), which presents constant systematic reformulations and with a lot of undescribed species waiting for scientific validation.

Pterygoplichthyini was considered sister group for Ancistrini (Ambruster, 2004) and cytogenetic data demonstrated 2n of 52 chromosomes in Pterygoplichthyini species (Alves *et al*., 2006). Previous cytogenetic studies in Ancistrini also showed a large number of species with 2n = 52 chromosomes, predominantly of meta and submetacentric chromosomes (Artoni, Bertollo, 2001; de Oliveira *et al*., 2006). Based on phylogenetic relationships of Hypostominae proposed by Lujan *et al*. (2015), and considering the

presence of 2n = 52 chromosomes in Pterygoplichthyini, the sister group for Ancistrini, Bueno *et al*. (2018) suggested that the putatively ancestral condition for Ancistrini is a diploid number of 52 chromosomes, from which chromosomal diversification occurred to explain the observed karyoptypic plasticity among studied species.

Ancistrus is the specious genus of Ancistrini and is widely distributed in South America (Ferraris, 2007; Armbruster, 2008; Lujan *et al*., 2013). In this tribe, only *Ancistrus* species presents a diversified condition from 2n = 52 chromosomes, with a higher frequency of acrocentric chromosomes (de Oliveira *et al*., 2007, 2008, 2009; Mariotto *et al*., 2009, 2011; Konerat *et al*., 2015; Favarato *et al*., 2016; Barros *et al*., 2017; Bueno *et al*., 2018). Cytogenetic data in *Ancistrus* revealed a diversity of 2n and karyotype formulas (details of available *Ancistrus* cytogenetic data can be found in Tab. 1), which range from 2n = 34 to 54 chromosomes (Mariotto *et al*., 2011). In addition, different heteromorphic sex chromosome systems are found in the genus, such as: XX/X0, XX/XY, XX/XY_1Y_2, ZZ/ZW and $Z_1Z_1Z_2Z_2$/ $Z_1Z_2W_1W_2$. In *Ancistrus,* with the exception of species with 2n = 52 and 54 chromosomes, it was suggested a reduction in 2n via Rb fusion events (de Oliveira *et al*., 2007, 2008, 2009; Mariotto *et al*., 2009, 2011; Konerat *et al*., 2015; Favarato *et al*., 2016; Barros *et al*., 2017) and structural chromosomal changes, such as inversions, translocations, deletions and duplications (Mariotto *et al*., 2011).

In addition to *Ancistrus*, other members of Loricariidae present species with 2n reduction via Rb fusions, when vestiges of interstitial telomeric sites (ITS) can be visualized in some karyotypes (Rosa *et al*., 2012; Errero-Porto *et al*., 2014; Favarato *et al*., 2016; Barros *et al*., 2017; Primo *et al*., 2017; Glugoski *et al*., 2018). Some of these Rb events were associated with the presence of EBRs inside 5S and 45S rDNAs sites, which triggered breaks and chromosomal reorganizations (Rosa *et al*., 2012; Barros *et al*., 2017; Primo *et al*., 2017; Glugoski *et al*., 2018). However, the presence of other repetitive DNA sequences, able of explaining the occurrence of other EBRs in the Loricariidae genomes, still remain uncertain (Primo *et al*., 2018).

Repetitive DNAs are organized as grouped blocks (microsatellites, mini-satellites, satellites and multigene families) or are dispersed (transposons and retrotransposons) on the chromosomes (Charlesworth, 1994). These repetitive sequences have been shown to be fundamental in studies related to genomic evolution (Maxon *et al*., 1983; Charlesworth *et al*., 1994; Vicari *et al*., 2010). Multigene families of rRNAs are composed of repetitions organized in tandem (Long, Dawid, 1980). They constitute two gene families with different loci in the karyotypes: the major rDNA 45S comprises the genes that encode the 18S, 5.8S and 28S rRNAs; while the minor rDNA codifies the 5S rRNA (Long, Dawid, 1980). In situ localization of rDNA sites showed that the dispersion and distribution of these repetitive DNAs may have contributed to genomic diversification and chromosomal remodeling among armored catfish (Rosa e*t al*., 2012; Errero-Porto *et al*., 2014; Barros *et al*., 2017; Primo *et al*., 2017; Glugoski *et al*., 2018).

Cytogenetic studies contribute to taxonomy by demonstrating difference in karyotypes of cryptic species (Vicari *et al*., 2006; Oliveira *et al*., 2016; Barbosa *et al*., 2017; Nascimento *et al*., 2018) or by detecting synonym species (Bellafronte *et al*., 2005). Given the morphological similarity present in some members of *Ancistrus* and the occurrence of a lot of scientific undescribed species in the scientific literature, the taxonomy of the group has been suffered numerous reformulations (de Oliveira *et al*., 2009; Lujan *et al*., 2015). In this study, the cytogenetic data of two species of *Ancistrus* were described and

TABLE 1 | Review of available *Ancistrus* cytogenetic data. "Unknown" means that the data was not available in the original manuscript. NOR: Nucleolar Organizer Region; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric; FN: Fundamental number. *one member of the homologous pairs with FISH markers.

Species	2n	FN	Karyotype formula	Sex chromosome system	rDNA 5S (pair)	rDNA 18S (pair)	Localization	Reference
Ancistrus cuiabae	34	68	20m+8sm+6st	Absent	Unknown	2	Arrombado bay-MT	Mariotto *et al.* (2009)
Ancistrus cuiabae	34	67	19m+8sm+6st+1a	Absent	Unknown	2	Arrombado bay-MT	Mariotto *et al.* (2009)
Ancistrus cuiabae	34	66	18m+8sm+6st+2a	Absent	Unknown	2 (NOR)	Arrombado bay-MT	Mariotto *et al.* (2009)
Ancistrus cuiabae	34	68	20m+8sm+6st	Absent	3, 6, 9	2	Arrombado bay-MT	Mariotto *et al.* (2011)
Ancistrus sp. Purus	34	68	♂21m+11sm+2st ♀20m+2sm+2st	XX/XY	3, 5, 12, 13	4	Purus river-AM	de Oliveira *et al.* (2009) Favarato *et al.* (2016)
Ancistrus sp. Catalão	34	68	22m+8sm+4st	XX/XY	3, 6, 7, 12	4	Lake Catalão-AM	Favarato *et al.* (2016)
Ancistrus sp. Trombetas	38	73	22m+8sm+5st+3a	Absent	Unknown	5 (NOR)	Trombetas river-PA	Oliveira *et al.* (2009)
Ancistrus n.sp. 1	38	76	30m/sm+8st	Absent	Unknown	5 (NOR)	São Francisco river-AC	Alves *et al.* (2003)
Ancistrus dubius *Ancistrus* sp. "Balbina"	♀38 ♂39	76 78	26m+10sm+2st 27m+10sm+2st	XX/XY_1Y_2	4	12	Barretinho stream-AM	de Oliveira *et al.* (2008) Favarato *et al.* (2016)
Ancistrus sp. 13	40	80	26m+10sm+4st	Absent	5, 15	18	Salgadinho stream-MT	Mariotto *et al.* (2011)
Ancistrus sp. 13	40	80	30m+6sm+4st	Absent	Unknown	18 (NOR)	Salgadinho stream-MT	Mariotto *et al.* (2013)
Ancistrus n.sp. 1	♂39 ♀40	78 80	33m+6sm 34m+6sm	XX/X0	Unknown	20 (NOR)	Vermelho river-GO	Alves *et al.* (2006)
Ancistrus cf. *dubius*	42	84	24m+10sm+8st	Absent	Unknown	16 (NOR)	Coxipó river-MT	Mariotto *et al.* (2006)
Ancistrus cf. *dubius*	42	84	24m+10sm+8st	XX/XY	4, 14, 16	16	Pari stream-MT	Mariotto *et al.* (2011)
Ancistrus cf. *dubius*	42	84	24m+10sm+8st	XX/XY	4, 14,16	16	Flechas stream-MT	Mariotto *et al.* (2011)
Ancistrus cf. *dubius*	42	84	24m+10sm+8st	XX/XY	4, 14, 16	16	Fundo stream-MT	Mariotto *et al.* (2011)
Ancistrus sp. Vermelho	42	78	26m+6sm+4st+6a	Absent	Unknown	20 (NOR)	Demeni river-AM	de Oliveira *et al.* (2009)
Ancistrus sp.	42	84	18m+16sm+8st	Absent	1, 10*	10	Criminoso stream-MS	Prizon *et al.* (2016)
Ancistrus cf. *dubius*	44	72	18m+10sm+16st/a	ZZ/ZW	Unknown	13 (NOR)	Serra das Araras stream-MT	Mariotto *et al.* (2004)
Ancistrus sp. 08	44	80	18m+10sm+8st+8a	ZZ/ZW	1, 13	13	Currupira river-MT	Mariotto *et al.* (2011)
Ancistrus maximus *Ancistrus* sp. Macoari	46	♂81 ♀82	18m+11sm+6st+11a 18m+12sm+6st+10a	XX/XY	19	19	Branco river-RR	Oliveira *et al.* (2006) Favarato *et al.* (2016)
Ancistrus abilhoai	48	90	22m+14sm+6st+6a	Absent	13	13	Iguaçu river-PR	Ribeiro *et al.* (2015)
Ancistrus ranunculus	48	82	♂20m+8sm+6st+14a ♀19m+9sm+6st+14a	ZZ/ZW	16	16	Xingu river-PA	de Oliveira *et al.* (2007) Favarato *et al.* (2016)
Ancistrus aguaboensis	50	80	16m+10sm+4st+20a	Absent	2, 21, 25	25	Ribeirão Bandeirinha river-GO	Present study
Ancistrus sp. 06	50	86	18m+10sm+8st+14a	Absent	21	21	Matrixã river-MT	Mariotto *et al.* (2011, 2013)
Ancistrus tombador	50	84	14m+12sm+8st+16a	Absent	Unknown	21 (NOR)	Preto river-MT	Mariotto *et al.* (2013)
Ancistrus cirrhosus	50	86	10m+14sm+12st+14a	Absent	1, 18, 23	17	Arroyo San Juan-Posadas (Argentina)	Prizon *et al.* (2017)
Ancistrus taunayi	50	92	22m+10sm+10st+8a	ZZ/ZW	21	24	Cascalho stream-SC	Konerat *et al.* (2015)
Ancistrus sp.	50	88	20m+12sm+6st+12a	Absent	4, 13, 15, 18	13	Unknown	Barros *et al.* (2017)
Ancistrus sp. "Mourão River"	50	92	12m+18sm+12st+8a	Absent	1, 14, 19, 20	12	Mourão river-PR	Prizon *et al.* (2017)
Ancistrus sp. "19 Stream"	50	92	♂11m+18sm+13st+8a ♀12m+18sm+12st+8a	XX/XY	1, 12, 15, 20, 22, 25	12	Stream 19-PR	Prizon *et al.* (2017)
Ancistrus sp. "Keller River"	50	92	♂11m+18sm+13st+8a ♀12m+18sm+12st+8a	XX/XY	1, 12, 15, 20, 25	12	Keller river-PR	Prizon *et al.* (2017)
Ancistrus sp. "SãoFrancisco Verdadeiro River"	50	94	14m+16sm+14st+6a	Absent	1, 15, 18, 21	18	São Francisco Verdadeiro river-PR	Prizon *et al.* (2017)

TABLE 1 | (Continued)

Species	2n	FN	Karyotype formula	Sex chromosome system	rDNA 5S (pair)	rDNA 18S (pair)	Localization	Reference
Ancistrus sp. "Ocoí River"	50	94	10m+18sm+16st+6a	Absent	18, 21, 22	18	Ocoí river-PR	Prizon *et al.* (2017)
Ancistrus sp. "São Francisco Falso River"	50	94	10m+18sm+16st+6a	Absent	11, 14, 18, 19	18	São Francisco Falso river-PR	Prizon *et al.* (2017)
Ancistrus cf. *multispinis*	52	84	16m+10sm+6st+20a	Absent	21, 25	24	Ribeirão Grande river-SP	Present study
Ancistrus sp.	52	76	12m+10sm+30st/a	Absent	13	3, 14	Angra dos Reis-SP	Reis *et al.* (2012)
Ancistrus n.sp. 2	52	90	10m+16sm+12st+14a	Absent	Unknown	15 (NOR)	Garuva river-SC	Alves *et al.* (2006)
Ancistrus sp. 04	52	82	16m+8sm+6st+22a	Absent	Unknown	22 (NOR)	Sepotuba river -MT	Mariotto *et al.* (2013)
Ancistrus n.sp. 2	52	84	32m/sm+20st/a	Absent	Unknown	24 (NOR)	Betari river-SP	Alves *et al.* (2003)
Ancistrus multispinnis	52	80	28m/sm+24st/a	Absent	Unknown	17 (NOR)	Itapocu river-SC	Alves *et al.* (2003)
Ancistrus aff. *dolichopterus* *Ancistrus* sp. "Piagaçu"	52	♂78 ♀79	16m+8sm+2st+26a 16m+9sm+2st+25a	ZZ/ZW	1, 5, 9, 14, 15, 20, 22, 24, 25, 26	26	Purus river-AM	de Oliveira *et al.* (2007) Favarato *et al.* (2016)
Ancistrus sp. Dimona	52	78	16m+8sm+2st+26a	Absent	Unknown	13 (NOR)	Fazenda Dimona stream-AM	de Oliveira *et al.* (2009)
Ancistrus sp. 4	52	82	16m+8sm+6st+22a	Absent	17, 25, 26*	22	Sepotuba river-MT	Mariotto *et al.* (2011, 2013)
Ancistrus dolichopterus *Ancistrus* sp. "Barcelos"	52	♂80 ♀79	12m+12sm+4st+24a 11m+12sm+4st+25a	$Z_1Z_1Z_2Z_2$/ $Z_1Z_2W_1W_2$	1, 2, 6, 8, 9, 15, 16, 18, 19, 20, 23, 24, 26	23	Demeni river-AM	de Oliveira *et al.* (2008) Favarato *et al.* (2016)
Ancistrus claro	54	84	14m+8sm+8st+24a	Absent	4, 19, 21	21	Coxipó river-MT	Mariotto *et al.* (2011, 2013)
Ancistrus sp. 03	54	84	14m+8sm+8st+24a	Absent	Unknown	21 (NOR)	Pari stream-MT	Mariotto *et al.* (2013)
Ancistrus sp. 01	54	84	14m+8sm+8st+24a	Absent	Unknown	21 (NOR)	Pipa stream-MT	Mariotto *et al.* (2013)

compared in order to add information to understand the chromosomal evolution in the genus and contribute to taxonomic and systematic aspects.

MATERIAL AND METHODS

Species analyzed. Twenty five specimens (13 males and 12 females) of *Ancistrus* cf. *multispinis* (Regan, 1912) from Ribeirão Grande river, Paraíba do Sul basin (Pindamonhangaba-SP, 22°47'8" S and 45°27"19" W) and 20 specimens (10 males and 10 females) of *Ancistrus aguaboensis* Fisch-Muller, Mazzoni, Weber, 2001 from Bandeirinha river, Tocantins basin (Formosa-GO, 15°19'25" S and 47°25'26" W) were cytogenetically analyzed. Specimens were deposited in the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) of the Universidade Estadual de Maringá, Maringá, Brazil (voucher numbers: *Ancistrus aguaboensis,* NUP 22305; *Ancistrus* cf. *multispinis*, NUP 22308).

Conventional cytogenetic procedures. The chromosomes were obtained from the air-drying method according to Bertollo *et al.* (2015). Detection of the constitutive heterochromatin was performed by C-banding according to Sumner (1972) and the nucleolar organizer regions (NORs) were detected by silver nitrate staining (Howell, Black, 1980). For karyotype assembly, homologs chromosomes were paired and grouped into metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a), according to Levan *et al.* (1964). To establish the fundamental number (FN), we

considered the m, sm and st chromosomes as two arms, and acrocentric chromosomes were considered as a single arm. About 30 cells with chromosomes in metaphase were analyzed for each species/method.

DNA extraction and isolation of repetitive DNAs. Genomic DNA was extracted from liver using Phenol-Chloroform method (Sambrook *et al*., 2001). Genomic DNA of both species was used as template in Polymerase Chain Reactions (PCRs) to obtain 5S rDNA sequences, using the following primers: 5Sa (5'- TACGCCCGATCTCGTCCGATC -3') and 5Sb (5'- CAGGCTGGTATGGCCGTAAGC -3') (Martins *et al*., 1999). The amplification reaction followed Barros *et al*. (2017) protocol. Agarose gel electrophoresis evidenced DNA fragments of approximately 1200 bp, which were isolated ("*PCR DNA and Gel Band Purification Kit*" - GE Healthcare) and cloned ("*InsTAclone PCR Cloning Kit"* - Promega), following the manufacturers' instructions. The 5S rDNA clones were sequenced (ABI-Prism 3500 Genetic Analyzer - Applied Biosystems). The obtained sequences were analyzed by BIOEDIT 5.0.9 (Hall, 1999), then submitted to an identity analysis on BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), Rfam (https://rfam.xfam.org/) and CENSOR (www.girinst.org/censor/index.php).

Fluorescence *in situ* hybridization (FISH). The FISH procedures were performed following Pinkel *et al*. (1986) protocol, with stringency ~77% (2.5 ng/μL probe, 50% formamide, 2x SSC, 10% dextran sulfate, at 37 °C for 16 h). It was used the following probes: 18S rDNA (Hatanaka, Galetti Junior, 2004), 5S rDNA (1200 bp DNA fragment amplified by PCR) and the general telomeric sequence of vertebrates (TTAGGG)n (Ijdo *et al*., 1991). The probes 5S rDNA and (TTAGGG)n were labeled by PCR using digoxigenin 11-dUTP (Jena Bioscience); 18S rDNA probe was labeled with biotin through the nick translation technique ("*Biotin16 NT Labeling Kit*" - Jena Bioscience). For signal detection, the antibodies Streptavidin Alexa Fluor 488 (Molecular Probes) and antidigoxigenin-rhodamine (Roche Applied Science) were applied. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI 0.2 μgmL^{-1}) in mounting medium Vectashield (Vector) and analyzed under an epifluorescence microscope Olympus BX51, coupled to the Olympus DP-72 camera with the DP2-BSW software. The best images were photographed, and karyotypes edited using Adobe Photoshop CS6.

RESULTS

Karyotypic description. *Ancistrus aguaboensis* presented 2n = 50 chromosomes, a karyotype formula arranged in 16m+10sm+4st+20a, FN = 80 and, without sex chromosome heteromorphism (Fig. 1A). C-banding revealed blocks of constitutive heterochromatin located on the centromeric and terminal regions of all chromosomes, in addition to one block on the pericentromeric region for the pair m2, on the interstitial long arm of the sm 9 and a large block on the terminal region of one member of the chromosome pair 18 (Fig. 1B). NORs sites were located on the short arms of acrocentric pair 25 (Fig. 1B, box).

Ancistrus cf. *multispinis* presented 2n = 52 chromosomes, a karyotype formula arranged in 16m+10sm+6st+20a, FN=84 and, no heteromorphism of sex chromosomes

was detected (Fig. 1C). The heterochromatin bands were located on the subterminal regions of the short arms of chromosomes pairs 1, 2, 3 and 10; in addition to blocks of heterochromatin on the subterminal regions of the long arms of chromosomes pairs 13, 17, 18 and 20, on the interstitial region of chromosome pair 23, and on one member of each homologs chromosome pairs 25 and 26 (Fig. 1D). NORs sites were visualized on the short arms of the acrocentric pair 24 (Fig. 1D, box).

***In situ* localization of rDNAs and telomeric sites.** FISH mapping of 5S rDNA probe in chromosomes of *A. aguaboensis* showed three chromosomal sites: in pericentromeric regions of the short arms of chromosome pairs 2 and 25, and a subterminal site on the acrocentric 21 (Fig. 2A). In situ localization of 18S rDNA sites evidenced signals on the subterminal region of the short arms of chromosome pair 25, syntenic with 5S rDNA sites (Fig. 2A). FISH mapping of (TTAGGG)n sequence showed telomeres regions marked (Fig. 2B), without ITS vestiges.

In situ localization of the 5S rDNA in *A.* cf. *multispinis* revealed sites on the subterminal regions of the short arms of acrocentric pairs 21 and 25, while 18S rDNA sites were located on the subterminal region of the short arms of acrocentric pair 24, which showed a variation in cistron size among the homologs (Fig. 2C). The FISH performed using telomeric sequence probes revealed only terminal chromosomal signals (Fig. 2D).

FIGURE 1 | Karyotypes of *Ancistrus aguaboensis* (**A–B**) and *Ancistrus* cf. *multispinis* (**C–D**) submitted do Giemsa staining (**A–C**) and C-banding (**B–D**). The chromosomes pairs with NORs sites are evidenced in the figure details (boxes). Bar = 10 µm.

Analysis of 5S rDNA sequences. The 1193 bp-long 5S rDNA sequence obtained from *A. aguaboensis* (GenBank accession no. MT018470) presented 95% identity with 5S rDNA gene of *Symphysodon* sp. (GenBank accession no. KP715274.1). This sequence shows an 120 bp open reading frame (ORF), 1073 bp of the non-transcribed spacer (NTS), an internal promoter comprising box A (47 - 59 bp), the intermediate element (IE) and the box C (78 - 95 bp) and, a poli-T cluster (downstream from transcribed region), a TATA-like region (-36 to -33), a GC box (-17 to -15) and a Citosin -1. The analyses using the CENSOR software revealed a 30 bp DNA fragment (1048 to 1078 bp) with 90.62% identity with the transposable element (TE) *Helitron* from *Oryza sativa (HELITRON3_OS)*.

The 1082 bp-long 5S rDNA sequence obtained from *A.* cf. *multispinis* (GenBank accession no. MT018471) showed 98% identity with 5S rDNA from *Symphysodon* sp. (GenBank accession no. KP715274.1). This sequence presents an 120 bp ORF and a 962 bp NTS. The internal promoter comprising box A (47 - 59 bp), IE and the box C (79 - 96 bp). The poli-T cluster (downstream from transcribed region), the TATA-like region (-33 to -26), GC box (-17 to -16) and a Citosin -1 were also detected. Analyses by CENSOR software revealed a 76 bp DNA fragment (736 to 812 bp) with 78.21% identity with the TE *hAT* from *Salmo salar* (*hAT-35N1_SSa)*. According to Rfam, the obtained 5S rDNAs have identity to 5S rRNAs between the segments 1-117, E-value = 4.2^{-19} for *A. aguaboensis* and E-value = 1.3^{-23} for *A.* cf. *multispinis.*

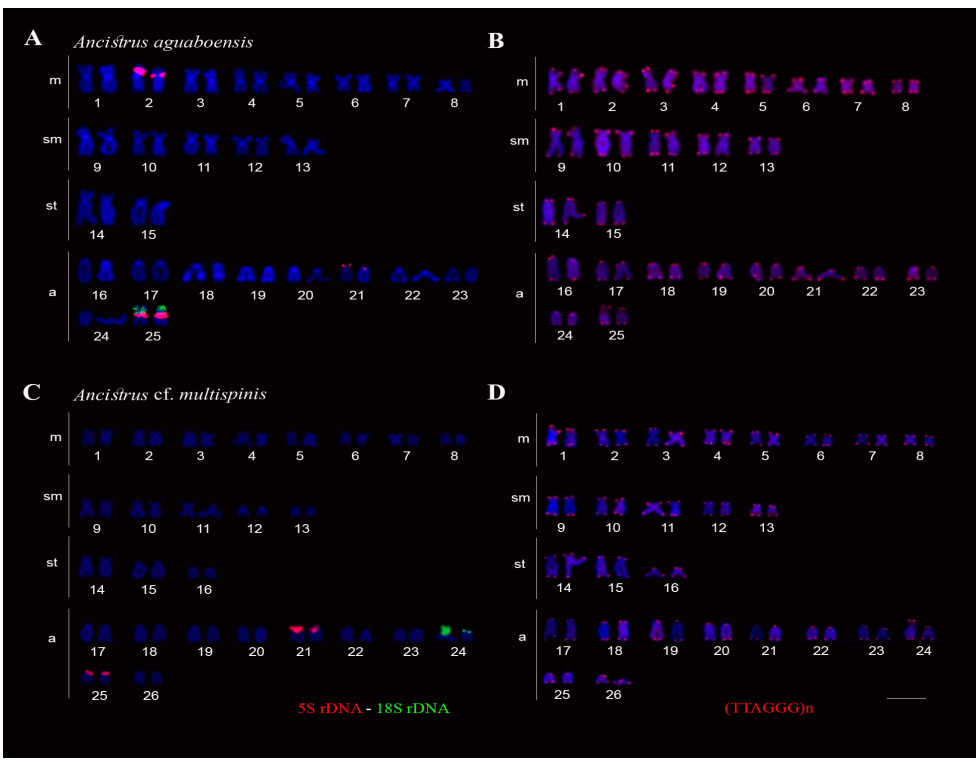

FIGURE 2 | Karyotypes of *Ancistrus aguaboensis* (**A–B**) and *Ancistrus* cf. *multispinis* (**C–D**) submitted to FISH using 5S rDNA, 18S rDNA and (TTAGGG)n probes. In (**A–C**), 5S rDNA (in red) and 18S rDNA (in green) sites; in (**B–D**), terminal red markers evidenced (TTAGGG)n sites. Bar = 10 µm.

DISCUSSION

Ancistrini and Hypostomini tribes show a wide range of 2n and karyotypes among their representatives (Bueno *et al*., 2012, 2018; Traldi *et al*., 2012; Lorscheider *et al*., 2018). Hypostomini presents high 2n values and diversified karyotypes, whilst in Ancistrini, numerous species of *Ancistrus* tend for the 2n reduction (Mariotto *et al*., 2011; Barros *et al*., 2017; Bueno *et al*., 2018). *Ancistrus* cf. *multispinis* exhibits 2n = 52 chromosomes with half of the chromosomes carrying st/a morphology, while *A. aguaboensis* has 2n = 50 chromosomes and 48% of its are st/a chromosomes. Bueno *et al*. (2018) showed that species of *Ancistrus* with 2n close to 52 chromosomes have about 50% of st/a chromosomes in their karyotypes, while species with smaller 2n have considerably lower amounts of chromosomes with this morphology. Corroborating this proposal, the occurrence of fusion events of st/a chromosomes leads to the formation of m/sm chromosomes, and consequent reduction of 2n in some species of *Ancistrus* (Mariotto *et al*., 2011; Favarato *et al*., 2016; Barros *et al*., 2017).

Ancistrus cf. *multispinis* specimens (Ribeirão Grande, Paraíba do Sul basin) analyzed in this study share 2n = 52 chromosomes with *A. multispinis* of the Itapocu river from the coastal basin (Tab. 1, Alves *et al*., 2003). However, differences in karyotype formulas between the two populations indicate microstructural chromosomal changes in allopatric populations. The absence of ITS vestiges also corroborates the indication of a conserved karyotype for the species. *Ancistrus aguaboensis* has its first karyotype description in this study, and 2n = 50 chromosomes suggests a numerical reduction by centric fusion. Aiming the location of ITS vestiges in fused chromosomes, few species of *Ancistrus* had the detection of (TTAGGG)n sequence probes in their genome (Favarato *et al*., 2016; Barros *et al*., 2017). In *Ancistrus* sp. (Barra Grande river, Paraná State, Ivaí basin), an ITS and 5S rDNA pseudogene were colocated on the metacentric pair 1 (Barros *et al*., 2017), as observed on the chromosome pair m2 of *A. aguaboensis*. In *A. aguaboensis*, no vestige of ITS was detected on the m/sm chromosomes, which may be a result of the loss of these ITS during the fusion event (Meyne *et al*., 1990). However, since EBRs can be reused in karyotype evolution (Pevzner, Tesler, 2003), the presence of a 5S rDNA site in the proximal region of pair m2 indicate its origin from centric fusion with consequent loss of (TTAGGG)n sequences.

The distribution of heterochromatin in karyotypes is a feature widely evaluated in fishes (Kantek *et al*., 2009; Vicari *et al*., 2010). The location of chromosome-specific heterochromatic blocks can be useful and collaborate in the recognition of Rb fusion points (Rosa *et al*., 2012; Barros *et al*., 2017; Glugoski *et al*., 2018), or in the recognition of heteromorphic sex chromosomes (de Oliveira *et al*., 2007, 2008, 2009; Mariotto *et al*., 2011; Konerat *et al*., 2015; Favarato *et al*., 2016; Schemberger *et al*., 2019). *Ancistrus* cf. *multispinis* and *A. aguaboensis* presented large heterochromatic blocks in some chromosomal pairs, however, with no indication of sex chromosome heteromorphisms. The presence of large heterochromatic blocks is a feature widely shared in *Ancistrus* (Mariotto *et al*., 2011; Konerat *et al*., 2015; Favarato *et al*., 2016), whereas the absence of large heterochromatin blocks has been described to be a plesiomorphic characteristic in Loricariidae (Ziemniczak *et al*., 2012).

A single chromosome pair carrying 45S rDNA (NOR) is a characteristic shared in all analyzed *Ancistrus* species (Bueno *et al*., 2018). *Ancistrus* cf. *multispinis* and *A. aguaboensis*

also had only a single pair carrying the 45S rDNA, but on different chromosomes. While *A.* cf. *multispinis* did not present co-located 45S/5S rDNAs, in *A. aguaboensis* these clusters were located in synteny in an acrocentric pair. In other *Ancistrus* species, the location of the 45S rDNA has also been shown to be widely varied (see Tab. 1). The 45S rDNAs sites in different chromosomal locations, in chromosomes pairs showing different sizes and morphologies and, in condition of synteny to the 5S rDNA, indicate several transpositions and/or other structural events involving the 45S rDNA in *Ancistrus*. Hence, the 45S rDNA site was considered an important cytotaxonomic marker in the group due to its wide chromosomal location variation, being in innumerous cases, species-specific (Mariotto *et al*., 2011).

While the 45S rDNA is located in a single chromosome pair in *Ancistrus*, the 5S rDNA can be present in a large number of chromosomal sites (ranging from 1 to 13 chromosome pairs) in the different species analyzed (see Tab. 1). Barros *et al*. (2017) proposed the dispersion of 5S rDNAs, and their pseudogenes, in subterminal regions of st/a chromosomes. EBRs located close to 5S rDNA pseudogenes could promote DSB and Rb fusion events (Barros *et al*., 2017). In fact, *A.* cf. *multispinis* and *A. aguaboensis* presented 5S rDNA sites in the subterminal regions of acrocentric pairs. In addition, *A. aguaboensis* presented 5S rDNA sequences at proximal region in the pair m2. Variations in the 5S rDNA location occurs in *Ancistrus* species, but the proximal 5S rDNA location in heterochromatic regions, with or without ITS vestiges, may explain a part of the Rb fusions present in the genus.

Previous cytogenetic studies in Trichomycteridae, Neoplecostominae and Hypoptopomatinae species proposed that the 45S/5S rDNA syntenic condition was present in the karyotypes of sister group for Loricariidae (Ziemniczak *et al*., 2012). Syntenic condition of rDNAs is widely visualized in karyotypes of Loricariidae representatives (Kavalco *et al*., 2004; Mariotto *et al*., 2011; Ziemniczak *et al*., 2012; Traldi *et al*., 2013; Bueno *et al*., 2014; Favarato *et al*., 2016; Barros *et al*., 2017). Analyzing the location of chromosome types and the position of rDNAs synteny sites in Ancistrini, it is more parsimonious to infer evolutionary recurrence indexes for this chromosome condition in this tribe. In fact, when evaluating the pattern of the karyotype distribution of rDNAs in *Ancistrus*, although it may be an allusive proposal, it is possible to corroborate the proposal of Barros *et al.* (2017), which rDNAs pseudogenes can organize EBRs and, these EBRs, have an evolutionary re-use to generate chromosome diversification in the group.

The analysis of the 5S rDNAs sequences of *A.* cf. *multispinis* and *A. aguaboensis* demonstrated that they have all the structures necessary for their function, although this analysis can be only predictive. Unlike the studies proposed by Barros *et al.* (2017) and Glugoski *et al.* (2018), 5S rDNA pseudogenes were not recovered in our analyzes. Pseudogenes are common in multigene families (Rebordinos *et al*., 2013). It is difficult to detect EBRs in multigene families, which depends of a large number of sequence analysis or the use of comparative genomics. Thus, the detailed assessment of the presence of EBRs in 5S rDNA pseudogenes/degenerated sequences still remains predictive. However, in *Ancistrus* species with $2n \leq 50$ chromosomes, the presence of 5S rDNA sites on m/sm chromosomes, originated from Rb fusion, could explain part of the chromosome diversification in the genus.

Ancistrus aguaboensis and *A.* cf. *multispinis* are found in sympatry and syntopy with species of *Harttia punctata* and *Harttia carvalhoi*, respectively. These species show

karyotype diversity, with the absence of sex chromosomes in *A. aguaboensis* and *A.* cf. *multispinis*, however with the presence of multiple sex chromosomes systems in *Harttia punctata* ($X_1X_1X_2X_2/X_1X_2Y$) and in *Harttia carvalhoi* (XX/XY_1Y_2) (Blanco *et al*., 2013, 2014). These armored catfishes inhabit small tributaries, which favors the isolation of populations, providing events of chromosome rearrangements that could be more easily fixed. This cytogenetic differentiation may be functioning as a reproductive barrier between species, a fact confirmed by the absence of hybrid species.

The wide karyotype diversification present in *Ancistrus* (Bueno *et al*., 2018) is compatible with the fact that the group is diverse and specious (Lujan *et al*., 2015). Chromosome rearrangements promote important differences in the genomic sets of species, which could lead to meiotic incompatibilities (Navarro, Barton, 2003). Chromosome segregation failure and the ensuing production of unviable gametes due to the accumulation of chromosomal rearrangements might play an important role in speciation (Navarro, Barton, 2003; Faria, Navarro, 2010). In the same way, the genetic differences accumulated in divergent *Ancistrus* species may have helped in the diversification of this evolutionary lineage.

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Larissa Glugoski: Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft.
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Stephane Schott: Methodology.
Marcelo R. Vicari: Methodology, Project administration, Supervision, Writing-review & editing.
Viviane Nogaroto: Methodology, Project administration, Supervision, Writing-review & editing.
Orlando Moreira-Filho: Funding acquisition, Investigation, Project administration, Supervision, Writing-review & editing.

ETHICAL STATEMENT

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COMPETING INTERESTS

The authors declare no competing interests.

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