Diversity and chromosomal evolution in the genus *Ancistrus* Kner, 1854 (Loricariidae: Ancistrini) from three hydrographic basins of Mato Grosso State, Brazil

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Cytogenetic analyses were carried out in 117 specimens of seven species of the genus *Ancistrus* from three hydrographic in Mato Grosso State: Paraguay, Araguaia-Tocantins and Amazon basins. Conventional cytogenetic techniques were used to obtain mitotic chromosomes. C-banding was performed to detect heterochromatic regions and silver nitrate staining was used to identify nucleolar organizer regions (Ag-NORs). The counted and paired chromosomes revealed diploid numbers ranging from 2n = 40 to 2n = 54 with karyotype formulae varying from FN = 80 to FN = 86. Single marks in distinct chromosomes identified the nucleolar organizer regions. The constitutive heterochromatin was scarce in the diploid number from 2n = 50 to 2n = 54 and conspicuous blocks were observed in a single species with 2n = 40 chromosomes. These data corroborate the hypotheses of reduction of diploid number in species with derived features such as presence of sex chromosomes and polymorphisms, besides allowing inferences about the evolutionary mechanisms and the ancestor karyotype that favored the diversification of this important genus in the tribe Ancistrini.

Foram realizadas análises citogenéticas de 117 espécimes do gênero *Ancistrus* de três bacias hidrográficas do estado de Mato Grosso: Paraguai, Araguaia-Tocantins e Amazônica, utilizando as técnicas de citogenética convencional para obtenção de cromossomos mitóticos, visualização de regiões heterocromáticas e regiões organizadoras de nucléolos. Os cromossomos pareados revelaram uma variação no número diploide de 2n = 40 a 2n = 54 e número fundamental de NF = 80 a NF = 86. As regiões organizadoras de nucléolos foram evidenciadas em um único par de cromossomos para todas as espécies e a heterocromátina é escassa nas espécies com números diploides elevados (2n = 50 a 2n = 54). Os blocos heterocromáticos mais evidentes foram observados nos pares portadores das AgRONs e em cromossomos da espécie com 2n = 40. Estes dados contribuem para a hipótese de redução do número diploide nas espécies que apresentam polimorfismos cromossômicos e cromossomos sexuais, além de contribuir para inferências sobre os mecanismos de evolução cariotípica que favoreceu a diversificação do gênero *Ancistrus*, o mais representativo na tribo Ancistrini.

Key words: AgNOR, Cytogenetic, Diploid number, Heterochromatin.

Introduction

The genus *Ancistrus* comprises small fish, up to 15 cm in total length, characterized by a large and compressed body covered with dermal plates, including some species with several long coetaneous tentacles (Burgess, 1989). Amongst Loricariidae, *Ancistrus* ranks second in the number of species (59 described ones), right after *Hypostomus* (Ferraris, 2007). The species of this genus are regarded as more derived than the other 26 genera reported in the tribe Ancistrini, a sistergroup of Pterygoplichthini (Armbruster, 1997, 2004).

The high karyotype diversity is found in *Ancistrus* species, whose diploid number ranges from 2n = 34 to 52 chromosomes, including simple and multiple sex chromosome systems and chromosomal polymorphisms (Artoni & Bertollo, 2001; Mariotto *et al.*, 2004; Mariotto & Miyazawa, 2006; de Oliveira *et al.*, 2008; Mariotto *et al.*, 2009).

Chromosomal rearrangements, such as fusions, pericentric and paracentric inversions, heterochromatin accumulation and/or translocations have played a major role in the karyotype evolution and diversification of some freshwater fish groups from Neotropical region (Giuliano-Caetano, 1998;

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Artoni & Bertollo, 1999; Kavalco *et al.*, 2005). According to Alves *et al.* (2003) and de Oliveira (2006), centric fusions are the main chromosomal rearrangement within *Ancistrus*, in which the reduction of diploid number seems to be an evolutionary trend. Nonetheless, other rearrangements, such as pericentric inversions and heterochromatin accumulation are also important for the chromosomal diversification and karyotype evolution of loricarids.

In the present work, cytogenetic analyses were carried out in different populations of seven *Ancistrus* species from three hydrographic basins in the state of Mato Grosso: Paraguay, Araguaia-Tocantins and Amazon.

Material and Methods

Cytogenetic studies were performed in 117 specimens from seven species of *Ancistrus* (Table 1) from three hydrographic basins in Mato Grosso State: Paraguay, Araguaia-Tocantins and Amazon basins. Voucher specimens were deposited in the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Paraná State, Brasil and identified by Dr. Claudio Zawadzki and coworkers (NUP 6827), and in the fish collection of the Museu de Ciências e Tecnologia da PUC-RS, Porto Alegre (PUC/RS), identified by Alexandre Cardoso and Tiago Carvalho (MCP 41969, 41973, 41974, 41976, 41977, 41978). Many of these are undescribed species and wait for a proper taxonomic description. The localities were georeferenced, approximately, according to I3geo map from Ministério do Meio Ambiente (MMA).

The technique for obtaining of mitotic chromosomes and conventional analysis followed Bertollo *et al.* (1978); C-banding was performed to identify heterochromatic regions according to Sumner (1972); and the nucleolar organizer regions (Ag-NORs) were identified by silver nitrate staining (Ag-NORs) (Howell & Black, 1980).

The chromosomes were organized into metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a), according to Levan *et al.* (1964).

Results

Different diploid numbers were observed among the analyzed species: 2n = 40 to 2n = 54 with karyotype formulae ranging from FN (Fundamental Number) =80 to FN = 86. The distinct diploid numbers, karyotype formulae and Ag-NOR locations are shown in Table 2.

Besides the variation shown in Table 2 and Fig. 2, the species from localities **a** to **f** presented small amount of heterochromatin as revealed by faint and reduced C-bands at interstitial and pericentromeric regions of some chromosomes. More evident heterochromatic blocks were detected only at the NOR-bearing pair in some metaphases. No cytogenetic differences were observed between males and females in these species based on conventional and C-banding analyses. An intraindividual polymorphism was observed in relation to NOR size, once larger and minor Ag-NOR marks were observed between homologues although all species presented single NORs with interstitial

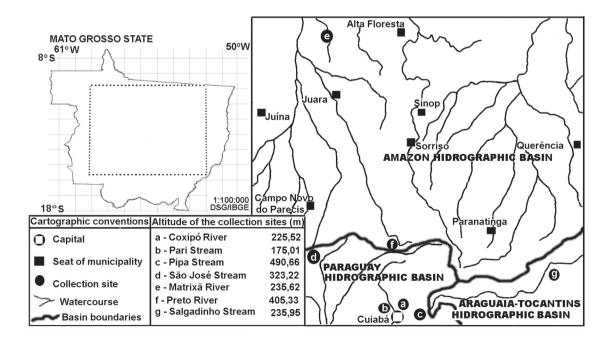


Fig. 1. Map of Mato Grosso State and sites crop (highlight-) showing Amazon, Araguaia-Tocantins and Paraguay hydrographic basins. Scale of original map 1:100.000.

Basin/Specie	Number of the indivuduals	Locality/County	Latitude and Longitude approximately	
Paraguay				
Ancistrus claro	11 M, 10 F	 a. Coxipó River/Chapada dos Guimarães 	15°21'S 55°57'W	
Ancistrus sp. 03	6 M, 6 F	b. Pari Stream /Cuiabá	15°36'S 56°12'W	
Ancistrus sp. 01	6 M, 7 F	 c. Pipa Stream/Serra de São Vicente 	15°41'S 55°29'W	
Ancistrus sp. 04	12 M, 15 F	d. São José Stream, Sepotuba River/Tangará da Serra	14°33'S 57°24'W 14°41'S 57°48'W	
Amazon				
Ancistrus sp. 06	9 M, 5 F	e. Matrixã River/Nova Monte Verde	10°03'S 57°36'W	
Ancistrus tombador	8 M, 5 F	f. Rio Preto/Diamantino	15°02'S 56°13'W	
Araguaia-Tocantins Ancistrus sp. 13	11 M, 6 F	g. Salgadinho Stream/Nova Xavantina	14°40'S 52°21'W	

Table 1. Analyzed *Ancistrus* species with their respective hydrographic basins and locality. M: males, F: females.

and terminal sites (Fig. 2, box). In those species with higher diploid numbers, 2n = 50 to 2n = 54, a larger number of small acrocentric chromosomes was identified, hindering a defined analysis. The constitutive heterochromatin is scarce, with small and usually faint blocks after C-banding (Fig. 3 a-f).

The specimens of *Ancistrus* sp.13 from \mathbf{g} site were highly differentiated from the others, with conspicuous heterochromatin blocks and reduced diploid number, 2n = 40 (Fig. 1g and g'). In some individuals, the long arms of a large subtelocentric chromosome were nearly entirely heterochromatic while a small heterochromatin segment was observed in the homologue.

Discussion

Previous analysis in the genus *Ancistrus* reported a high chromosomal plasticity in relation to diploid number (2n = 3 4, 38, 40, 42, 44, 46, 48, 50, 52, and 54); presence of five out of the seven sex chromosome systems known for freshwater fish, structural and morphology polymorphism in 18S rDNA and diversity 5S rDNA (Artoni & Bertollo, 2001; Alves *et al.*, 2003; Mariotto *et al.*, 2004; Mariotto & Miyazawa, 2006; de Oliveira *et al.*, 2008; Mariotto *et al.*, 2009; de Oliveira *et al.*, 2009, Mariotto *et al.*, 2011 and present study).

Ancistrus is characterized by a divergent karyotype evolution in contrast with other species within the tribe, such as species of the genera *Baryancistrus*, *Peckoltia*, *Hemiancistrus*, *Panaque*, *Parancistrus*, and *Lasiancistrus* (Artoni & Bertollo, 2001; Souza, 2003; de Oliveira *et al.*, 2006; Mariotto, 2009). It also differs from closely related taxonomic

groups, like most species analyzed in the subfamily Hypoptopomatinae (Andreata *et al.*, 1992, 1993, 2006) and in the tribe Pterygoplichthini (Alves *et al.*, 2006).

Chromosomal and heterochromatic heteromorphisms, including XX/XY and ZZ/ZW sex chromosome systems, were identified in four out of the five *Ancistrus* species with cytogenetic studies for the Pantanal region (Mariotto *et al.*, 2004; Mariotto & Miyazawa, 2006).

According to Gold et al. (1990), a small amount of heterochromatin is a basal trait for teleosteans, including Siluriformes fishes. This statement supports the hypothesis that the karyotypes of Ancistrus from Paraguay basin presenting more heterochromatin segments and sex chromosome systems would be more derived, such as reported in Ancistrus cf. dubius (Mariotto et al., 2004; Mariotto & Miyazawa, 2006), a species in which heterochromatinization of one chromosome in the heteromorphic pair seems to have played a major role in the fixation of sex chromosome systems. According Artoni & Bertollo (1999) and Almeida-Toledo et al. (2001), the heterochromatin accumulation and changes in its distribution on chromosomes might favor the chromosomal heteromorphism and are usually associated with the origin of sex chromosomes in fish. However, in some Ancistrus species, the origin of sex chromosomes seems to be unrelated to constitutive heterochromatin, as observed in species from Amazon basin (de Oliveira et al., 2008).

Besides *Ancistrus*, other Loricariidae genera also present species with sex chromosome systems, such as *Hypostomus* (Michele *et al.*, 1977; Artoni *et al.*, 1998), *Microlepidogaster* and *Pseudotocinclus* (Andreata *et al.*, 1992, 1993),

Table 2. Karyotype data of analyzed *Ancistrus* species in increasing diploid number order.

Specie	Locality	2n	NF	Karyotypic formula	AgNORs
Ancistrus claro, Ancistrus sp. 01, Ancistrus sp. 03	a, b, c	54	84	14m, 8sm, 8st, 24a	21° a
Ancistrus sp. 04	ď	52	82	16m, 8sm, 6st, 22a	22° a
Ancistrus sp. 06	e	50	86	18m, 10sm, 8st, 14a	21° a
Ancistrus tombador	f	50	84	14m, 12sm, 8st, 16a	20° a
Ancistrus sp. 13	g	40	80	30m, 6sm, 4st	18° sm

Loricariichthys (Scavone & Júlio Jr., 1995), Hemiancistrus (de Oliveira et al., 2006) and Harttia (Centofante et al., 2006).

However, in the present study, the species located at higher altitude differed from those located in lower places and/or regions with high sediment flow. The cytotypes of species with 2n = 50, 52 and 54 chromosomes (Fig. 2) are in more

elevated areas in relation to others, such as the rivers and streams from Tangará da Serra, Serra de São Vicente, Chapada dos Guimarães, and small tributaries from Amazon basin, northern Mato Grosso (Fig. 1); regarded as geomorphologic ancient areas when compared to Cuiabá depression and the region close to Nova Xavantina (see Ribeiro, 2006). The species

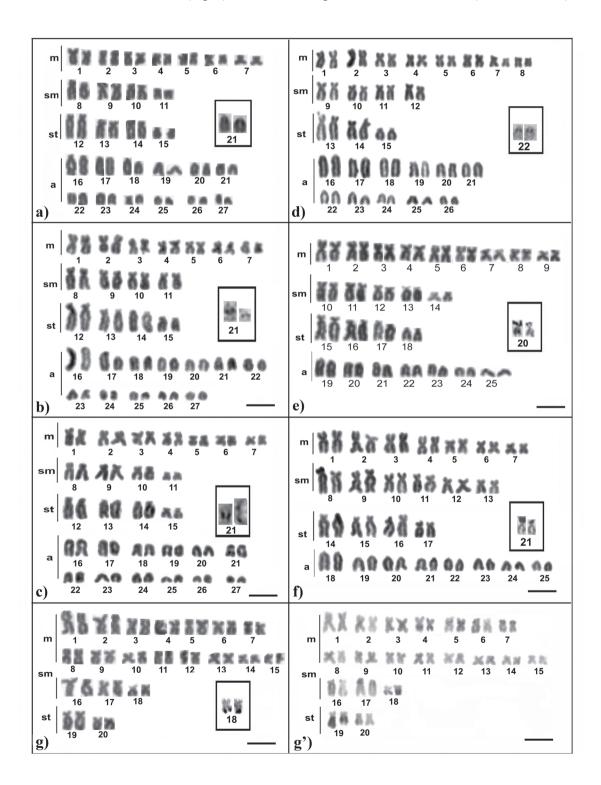


Fig. 2. Karyotypes of Ancistrus with AgRONs in boxes (a-g); C band of Ancistrus sp. 13 (g'). Bars 10μm.

with 2n = 34, 40 and 2n = 42 chromosomes occur in the Cuiabá depression, Nova Xavantina and Pantanal, areas susceptible to increased sedimentation (Mariotto & Miyazawa, 2006; Mariotto *et al.*, 2009). Most likely, the species with higher diploid numbers (2n = 50 to 2n = 54), bearing small amounts of heterochromatin and free from intraspecific polymorphism, are those characterized by plesiomorphic chromosomal features in the genus *Ancistrus*.

Chromosomal rearrangements, including fusions, pericentric inversions, transpositions, and heterochromatin accumulation, were probably responsible in determining the derived karyotypes, sex chromosomes systems and polymorphisms observed in species from Cuiabá depression. The chromosomal fusions and pericentric inversions are supported by the identification of fundamental numbers (FN) from 80 to 86 in the analyzed species, especially when species with striking differences in the diploid number are considered, such as *Ancistrus claro* (2n = 54) and *Ancistrus* sp. 13 (2n = 40).

The fixation of chromosomal changes in small and low vagile populations (Oliveira *et al.*, 1988), as typical of most Loricariidae, is once again reported in species of the genus *Ancistrus*. The basal diploid number of 2n = 54 chromosomes proposed for Loricariidae (Artoni & Bertollo, 2001) seems to corroborate the inferences about the putative ancestor karyotype of 2n = 54 chromosomes in the tribe Ancistrini, as observed in *Ancistrus claro* (present study) and in the genus *Lasiancistrus* (Mariotto, 2009), besides species from Hypoptopomatinae and

Pterygoplichthini, phylogenetically related to Ancistrini.

Armbruster (1997, 2004) places *Ancistrus* as the most phylogenetically derived genus in the subfamily. The chromosomal data obtained in the present work confirms this hypothesis once this genus is characterized by divergent karyotypes, regarding either chromosomal structure or diploid number, leading to higher karyotype diversity than other Ancistrini representatives.

Since species bearing multiple sex chromosomes (de Oliveira *et al.*, 2008), as reported in Amazon basin, and simple sex chromosome systems have already been identified in *Ancistrus* (Mariotto *et al.*, 2004; Mariotto & Miyazawa, 2006; de Oliveira *et al.*, 2006, 2007; Mariotto, 2009) according to each locality, a fixed pattern of sex heterogamety cannot be defined, thereby confirming the recurrence and the homoplasic character of sex chromosomes in the tribe Ancistrini.

The nucleolar organizer regions (NORs) in *Ancistrus* are highly conserved, comprising single marks in all species analyzed so far (Mariotto *et al.*, 2004; Mariotto & Miyazawa, 2006; de Oliveira *et al.*, 2008; Mariotto *et al.*, 2009 and present study) without any case of multiple NORs in the genus. The few cases of multiple NOR systems were reported in other genera and species of the tribe Ancistrini like *Peckoltia* sp. 1 and *Peckoltia* sp. 2 (Souza, 2003) and *Hemiancistrus spilomma* (de Oliveira *et al.*, 2006).

Single NORs at interstitial position are putatively a primitive feature within Ancistrini, as proposed by Artoni & Bertollo (2001),

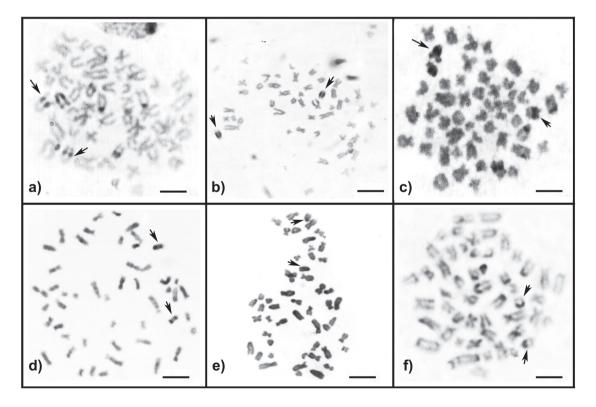


Fig. 3. Metaphases of *Ancistrus* with C band showing chromosomes NOR positive with large heterochromatin block (arrows). Bars 10 μm.

Alves et al. (2003), Mariotto et al. (2004), Mariotto & Miyazawa (2006), de Oliveira et al. (2006, 2007), Mariotto et al. (2009), Mariotto et al. (2011) and the present study. Nevertheless, NORs located at terminal position of chromosomes are observed in some species, as verified in Ancistrus n.sp. 2 and Ancistrus multispinnis (Alves et al., 2003); Hemiancistrus spinosissimus (de Oliveira et al., 2006); Ancistrus sp. "Piaguçu", Ancistrus sp. 1 "Balbina", Ancistrus sp. 2 "Barcelos" (de Oliveira et al., 2007); Ancistrus sp. 06, Ancistrus tombador, Lasiancistrus cf. schomburgkii, and Lasiancistrus sp. 1 (Mariotto, 2009 and present study). Most likely, the difference in NOR size verified in several species of the tribe Ancistrini is a feature related to a common ancestry, once it is shared by many taxa in the family Loricariidae. As for the chromosomal location, it corroborates the hypothesis of chromosomal rearrangements.

The present results show the remarkable plasticity in the genome organization of chromosomes in *Ancistrus* species from 2n = 40 to 2n = 54, that ultimately reflects their morphological plasticity, with representatives inhabiting fastflowing streams (plateau regions) up to flooded areas (plain regions), thus showing their adaptability to new niches. This evidence suggests speciation events once 13 cytotypes have already been reported in this group, which probably refer to new undescribed species.

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