

Chromosome polymorphism in *Ancistrus cuiabae* Knaack, 1999 (Siluriformes: Loricariidae: Ancistrini)

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Cytogenetic and FISH analyses were performed in 30 *Ancistrus cuiabae* specimens from a bay near the town of Poconé, in the Pantanal of Mato Grosso, Brazil. The observed diploid number was $2n = 34$ chromosomes for both sexes and three distinct karyotypic formulae were found, namely cytotype A (20m, 8sm, 6st, Fundamental Number/FN = 68; 6 males and 11 females), cytotype B (19m, 8sm, 6st, 1a, FN = 67; 8 males and 4 females) and cytotype C (18m, 8sm, 6st, 2a, FN = 66; a single male). NORs's analyses showed that these regions were located in distinct sites on the NOR-bearing chromosome pair, according to cytotypes. Thus, in cytotype A, NORs were located in the terminal region of the short arm of the second metacentric chromosome pair; in cytotype B, they were detected in the short arm of the metacentric chromosome and interstitially on the acrocentric chromosome and, in cytotype C, NORs were observed in the interstitial region of the acrocentric chromosome pair. C-positive heterochromatic bands were adjacent to the rDNA sites in the corresponding chromosomes. Thus, the chromosomal polymorphism of *A. cuiabae* was probably originated through a pericentric inversion in chromosome pair n° 2 involving the NOR sites, which represents a novelty in the Ancistrini tribe. The results also broaden the knowledge of the chromosomal evolution in *Ancistrus*, the most derived genus of the Ancistrini tribe.

Foram analisados, com técnicas convencionais de citogenética e FISH, 30 exemplares da espécie *Ancistrus cuiabae* da baía Arrombado, próximo a Poconé, Pantanal do Mato Grosso. Foram observadas metáfases com número diploide $2n = 34$ cromossomos para ambos os sexos e três fórmulas cariotípicas distintas, aqui denominadas de citótipo A, verificado em 06 machos e 11 fêmeas (20m, 8sm, 6st, Número Fundamental, NF = 68); citótipo B, em 08 machos e 04 fêmeas (19m, 8sm, 6st, 1a, NF = 67) e citótipo C em apenas 01 macho (18m, 8sm, 6st, 2a, NF = 66). As NORs confirmaram os distintos citótipos verificados, além de evidenciar que os cromossomos portadores de rDNA são os que representam o polimorfismo na espécie *Ancistrus cuiabae*. No citótipo A, as NORs foram verificadas na região terminal do braço curto do segundo par de cromossomos metacêntricos; no citótipo B, foram evidenciadas no segundo par, heteromórfico, no braço curto do cromossomo metacêntrico e intersticial no seu homólogo acrocêntrico; no citótipo C as NORs foram observadas na região intersticial num par de cromossomos acrocêntricos. A análise da heterocromatina constitutiva evidenciou blocos discretos adjacentes ao rDNA no segundo par de cromossomos de ambos os citótipos. Uma provável inversão pericêntrica é a hipótese proposta para a origem deste polimorfismo na espécie *Ancistrus cuiabae*. Estes resultados ampliam o conhecimento sobre o gênero *Ancistrus*, o mais derivado da tribo, contribuem para o conhecimento sobre este grupo de peixes e para inferir sobre a evolução cromossômica dos Ancistrini.

Key words: Cytogenetics, NOR, Pericentric inversion, Chromosomal evolution.

Introduction

The Ancistrini tribe is composed of 29 genera, totaling 217 nominal species, with four genera being represented by monotypical species (Fisch-Muller, 2003). Considering only the Brazilian hydrographic system, this tribe comprises 21 genera and 78 nominal species found mainly in the Amazonian

rivers (Sarmiento-Soares & Ingenito, 2007). *Ancistrus* corresponds to the most diversified genus, presenting 62 described species (Bifi *et al.*, 2009). This group is characterized by a smooth snout and may present a sexual dimorphism in many species, with numerous long and forked barbells in males and sparse and short ones in females. However, new species are being recognized and waiting to be described. In

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fact, the number of described Loricariidae species has risen significantly as a whole in the last years, due to the intensification and refinement of the studies in this family.

Cytogenetics and molecular genetics have been useful tools for clarifying doubts concerning the identification of some species, as well as for proposing phylogenies for a few fish groups. In *Ancistrus*, these analyses have showed a prominent numerical chromosome diversity, from $2n = 34$ in *Ancistrus* sp. 1 and *Ancistrus* sp. 2 in Amazonian rivers (de Oliveira, 2006), to $2n = 52$ in *Hemiancistrus* sp. and *Panaque* cf. *nigrolineatus* (Artoni & Bertollo, 2001).

Among Neotropical fish, chromosomal variations directly related to the number and/or size of the nucleolar organizer regions (NORs) are known for many groups, such as Gymnotiformes (Foresti *et al.*, 1981; Almeida-Toledo, 1985), Parodontidae (Moreira-Filho *et al.*, 1984), and Serrasalminae (Galetti *et al.*, 1985), among others. Recently, these variations have been confirmed by the use of fluorescent *in situ* hybridization (FISH) with 18S rDNA probes (Kavalco *et al.*, 2005; Mantovani *et al.*, 2005; Pazza *et al.*, 2006). However, structural polymorphisms due to other chromosomal rearrangements such as inversions and centric fusions/fissions are not very common among Neotropical fish, being known mainly among Characiformes (Giuliano-Caetano & Bertollo, 1988; Cestari & Galetti Jr., 1992; Centofante *et al.*, 2002; Jorge & Moreira Filho, 2004; Pazza *et al.*, 2006). In Siluriformes, numerical and/or structural polymorphisms were observed in a few Pimelodidae (Dias & Foresti, 1993; Vasconcelos & Martins-Santos, 2000), Heptapteridae (Vissoto *et al.*, 2001; Kavalco *et al.*, 2004), Hypoptopomatinae (Andreati *et al.*, 1994), Loricariinae (Giuliano-Caetano, 1998), and Trichomycteridae species (Borin & Martins-Santos, 2000).

In the present work, it is described a structural polymorphism in the NOR-bearing chromosomes of *Ancistrus cuiabae*, revealed by conventional cytogenetic techniques and fluorescent *in situ* hybridization with an 18S rDNA probe.

Material and Methods

Fifteen male and fifteen female specimens of *Ancistrus cuiabae* Knaack, 1999, were cytogenetically studied. The site collection of this species is situated 36 km away from Poconé (approximately 16°21'21"S 56°27'55"W), in the State of Mato Grosso, Brazil. This location, regionally known as Arrombado, has the characteristics of a bay in the Pantanal region, because it connects to the Bento Gomes River (Paraguay basin) in the flooding season (Fig. 1). There is still no record of this species occurring in any another locality. Voucher specimens were deposited in the fish collection of the Museu de Ciências e Tecnologia da PUC-RS, Porto Alegre (MCP41979).

The chromosome preparations were obtained by the air drying method (Bertollo *et al.*, 1978). The C-positive band heterochromatin and the Nucleolar Organizer Regions (Ag-NORs) were detected according to Sumner (1972) and Howell & Black (1980). The chromosomes were organized into four morphological groups (m = metacentric, sm = submetacentric,

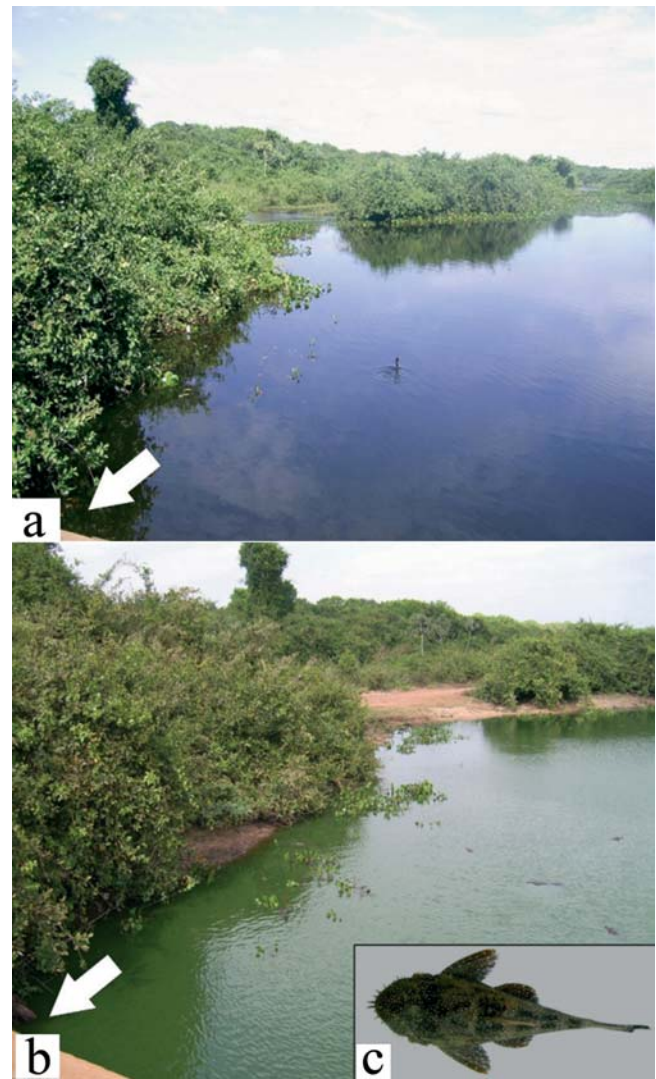


Fig. 1. (a-b) Partial view of the Arrombado bay and the sampling site collection (arrow). (c) *Ancistrus cuiabae* specimen with 110 mm.

st = subtelocentric, a = acrocentric) in decreasing order of size, according the arm ratios (Levan *et al.*, 1964). Fluorescent *in situ* hybridization (FISH) followed the protocol described by Pinkel *et al.* (1986), using 18S rDNA probes (Hatanaka & Galetti Jr., 2004).

Results

All the specimens evidenced the diploid number $2n = 34$ chromosomes in both sexes, in a sample greater than 1,000 analyzed cells. However, three karyotypic formulae were observed, here named cytotypes A, B and C.

Cytotype A, found in 6 males and 11 females, was characterized by 20m, 8sm and 6st chromosomes and FN (fundamental number or number of chromosome arms) equal to 68 (Fig. 2a). Ag-NORs were observed in the terminal region of the short arms of the second chromosome pair, characterized as metacentric. Fluorescent *in situ* hybridization results agreed

with the Ag-NORs locations (Fig. 3a, box).

Cytotype B, detected in 8 males and 4 females, was characterized by 19m, 8sm, 6st, 1a chromosomes and FN = 67 (Fig. 2b). Ag-NORs were evidenced in the terminal region of the short arm of a single metacentric chromosome and the interstitial region of the long arm of a single acrocentric chromosome of the karyotype, which was also corroborated by the FISH data with the 18S rDNA probe (Fig. 3b, box).

Cytotype C, detected in a single male, presented 18m, 8sm, 6st, 2a chromosomes and FN = 66. (Fig. 2c). In this case, the Ag-NORs were located in the interstitial region of the long arms of the acrocentric pair, characteristic of this cytotype (Fig. 3c, box).

All cytotypes presented a discrete amount of heterochromatin in the chromosomes, with more prominent C-positive bands adjacent to the NOR regions (Fig. 3 a,b,c).

Discussion

Loricariidae fish shows a very large variation of the chromosome number as a whole, which ranges from $2n = 80$ in a *Hypostomus* species (Artoni & Bertollo, 1996), to $2n = 34$ in *Ancistrus* species (de Oliveira, 2006; present work). Besides the chromosome number, the karyotypic formula is also variable, showing a differentiated karyotypic evolution throughout the speciation process of this family. Oliveira *et al.* (1988) considered that the fixation of different chromosome rearrangements would be more likely in populations with reduced vagility, as is the case of some Loricariidae species, thus promoting karyotypic variability.

Alves *et al.* (2005) suggested that the karyotypic diversity of Loricariidae could be exclusive to *Hypostomus*, since other loricariid groups seem to be characterized by a relatively conserved diploid number. This opinion was also shared by Lara (1998), Artoni & Bertollo (2001) and Souza (2003). However, as new studies were performed in Ancistrini it was pointed out that the chromosomal diversity go beyond the previously supposition for this group. Indeed, the Ancistrini tribe is characterized by a chromosomal diversification, especially in the genus *Ancistrus* where distinct karyotypic structures indicate a probable species complex (Mariotto & Miyazawa, 2006), besides the occurrence of simple and multiple sex chromosome systems (Mariotto *et al.*, 2004; Mariotto & Miyazawa, 2006; de Oliveira *et al.*, 2007, 2008), as well as simple or multiple and interstitial or terminal NOR systems (Alves *et al.*, 2003; Mariotto *et al.*, 2004; Souza *et al.*, 2004; Mariotto & Miyazawa, 2006; de Oliveira *et al.*, 2006).

The data from the present study corroborate the karyotypic diversity of *Ancistrus*. In fact, the analysis of the three cytotypes detected in *A. cuiabae* evidenced a case of structural chromosome polymorphism, associated to the NOR-bearing chromosome pair. This way, while in cytotype A this pair is metacentric, in cytotype B it is composed of a metacentric and an acrocentric chromosome, and in cytotype C by an acrocentric pair. Cytotype A was the most frequent in the studied sample and, probably, it corresponds to the

standard karyotypic form of the species, being present in 57% of the specimens. On the other hand, cytotype B was detected in 40% of the specimens, and cytotype C in only 3% of the sample. Therefore, it is likely that the observed

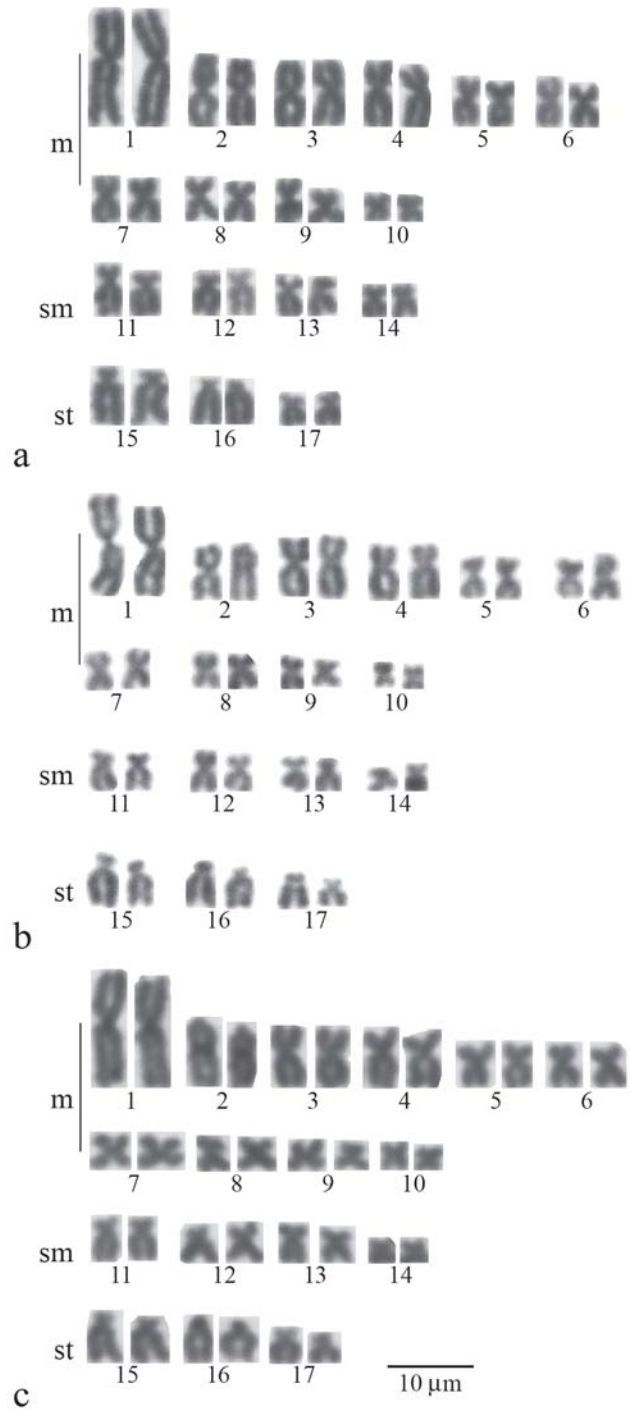


Fig. 2. Karyotypes of *Ancistrus cuiabae*, after conventional Giemsa staining, showing the polymorphic second chromosome pair. (a) Cytotype A with two metacentric chromosomes, (b) cytotype B with a metacentric and an acrocentric chromosome, and (c) cytotype C with two acrocentric chromosomes.

polymorphism is due to pericentric inversions that, at the same time, modified the chromosome form (metacentric/acrocentric) and the location of the NORs on the chromosomes (terminal/interstitial). Hence, cytotype A would

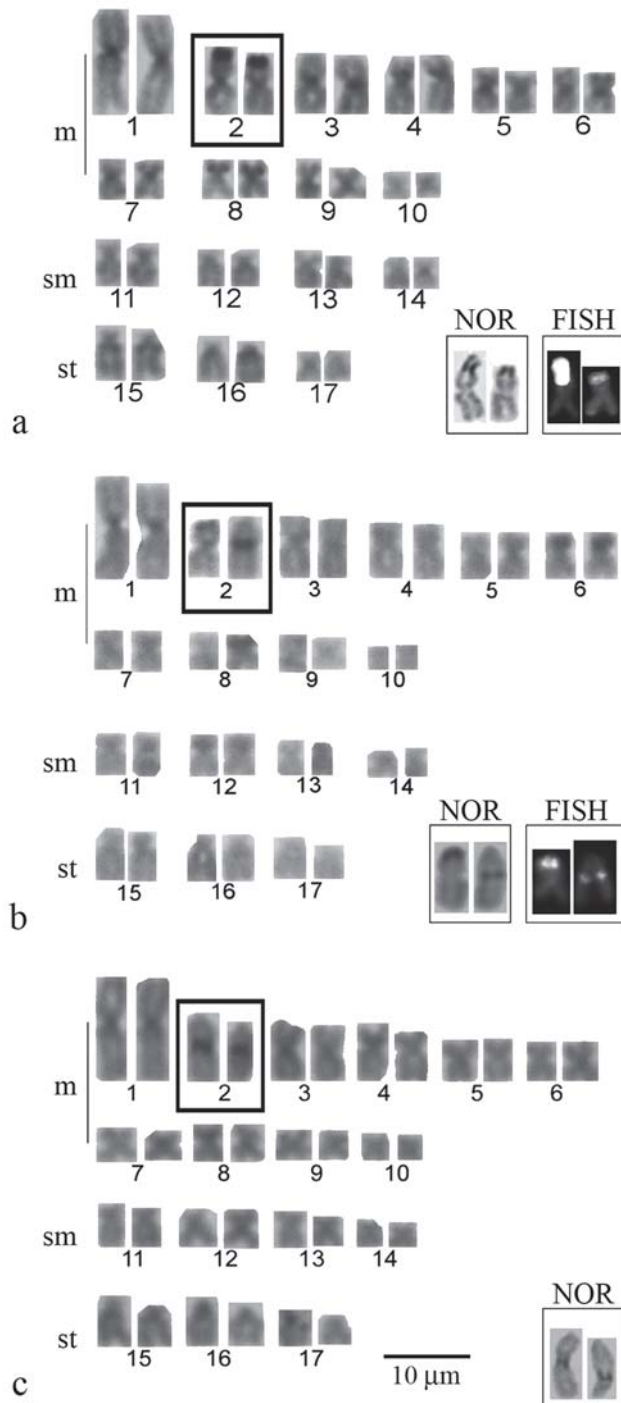


Fig. 3. Karyotypes of *Ancistrus cuiabae*, after sequential C-banding, evidencing the C-positive heterochromatin in the cytotypes A (a), B (b) and C (c). In the boxes, chromosomes from the second pair after silver staining and fluorescent *in situ* hybridization (FISH), showing the Ag-NOR and the 18S rDNA sites, respectively.

correspond to the basic homozygotes cytotype B to the structural heterozygotes and cytotype C to the structural homozygotes in the population.

Chromosome polymorphism due to pericentric inversions have already been observed in some fish groups. A pericentric inversion concerning the first karyotypic pair was detected in *Serrasalmus spilopleura* (Serrasalminae) from the Catalão Lake (Amazon basin), with absence of the structural homozygotes (Centofante *et al.*, 2002). Variations concerning the number of the acrocentric chromosomes in *Apareiodon affinis* (Parodontidae) from the lower Paraná River (Argentina) were also probably due to pericentric inversions, since the diploid number ($2n = 54$) was constant in the population (Jorge & Moreira-Filho, 2004). Pericentric inversions were also considered in order to explain another few cases of structural polymorphisms, as in *Hoplerythrinus unitaeniatus* (Erythrinidae) from the Negro River, in the Amazon basin (Giuliano-Caetano & Bertollo, 1988). On the other hand, very few cases of paracentric inversions are well known in fishes, possibly given the greater difficulty of detection by conventional chromosome analyses. In *Apareiodon piracicabae*, for example, this rearrangement was considered to explain the occurrence of syntenic double NORs in a chromosome pair of this species (Moreira-Filho *et al.*, 1984; Jesus & Moreira-Filho, 2000).

A simple NOR system, as present in *A. cuiabae*, seems to constitute a plesiomorphic condition in Ancistrini, which also occurs in the Hypoptopomatinae, considered a close external group. Therefore, a multiple NOR system, present in a few species of this tribe, such as *Peckoltia* sp. 1, *Peckoltia* sp. 2 (Souza, 2003) and *Hemiancistrus spilomma* (de Oliveira *et al.*, 2006), may represent a derived characteristic for this group. Loricariidae generally possess little heterochromatin, as also found in *A. cuiabae*, which seems like an ancestral condition for this group. A few exceptions may occur, as in *Hypostomus emarginatus*, where larger blocks of C-positive bands were verified and coinciding with a higher diploid number, this latter also considered as a derived condition (Artoni, 1996). In *A. cuiabae*, the C-positive heterochromatin presented a pericentromeric distribution, including the acrocentric chromosomes, which corroborates the role of pericentric inversions originating the polymorphism observed in the population.

Porto-Foresti *et al.* (2004), detected a NOR polymorphism in *Oncorhynchus mykiss* from controlled breeding, suggesting that heterozygote individuals would have a greater adaptive value in relation to the homozygote ones. Even though the reduced frequency of cytotype C may be due to a sampling bias, it is tempting to correlate the distinct frequencies of the *A. cuiabae* cytotypes with a differential fitness, as suggested for *O. mykiss*. It is probable that *A. cuiabae* is restricted to the studied collection site, since it has not been found in other nearby locations or even in rivers of different hydrographic basins. During the dry season, the water level of the bay where the population resides is drastically reduced. In this period, the *A. cuiabae* population must suffer an intensive

selective pressure from inter-specific competition or predation inside the bay, which loses its connection with the Bento Gomes River. A possible greater adaptability/resistance for such conditions, presented by a specific karyotypic form, could have an important role for the survival of the species, guaranteeing its maintenance in an inhospitable habitat. This is an interesting hypothesis that could be tested in further comparative studies, by comparing the cytotypes frequencies between the favorable and the non-favorable conditions during the dry season.

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