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Cytogenetic description of *Ancistrus abilhoai* (Siluriformes: Loricariidae) from Iguaçu River basin, southern Brazil

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ABSTRACT. The Iguaçu River basin is a tributary to the upper Paraná River in southern Brazil, and is considered an important aquatic ecoregion that, although having few species of fish, 51-71% of these are apparently endemic. *Ancistrus abilhoai* is one of three recently described species for this basin and is currently considered endemic to the basin. In this study, we present the chromosomal structure of two populations of *Ancistrus abilhoai* one collected in the Iguaçu River, in Paraná State, and another collected in the Timbó River, a tributary of the Iguaçu River, in the State of Santa Catarina. Karyotype analyzes were performed in 11 specimens from the Iguaçu River (four females and seven males) and 12 specimens (all males)

from Timbó River, revealing 2n = 48 chromosomes with a karyotype formula of 22m + 14sm + 6st + 6a in both populations. Analysis of active nucleolar organizer regions (Ag-NORs) and fluorescent *in situ* hybridization (FISH) with 18S rDNA probes revealed the submetacentric pair 13 bearing marks at terminal positions on the short arms. Considered as plesiomorphic chromosomal markers in Loricariidae, asynteny 18S and 5S rDNA, and small amounts of heterochromatin were observed. In this study, the first chromosomal data of *A. abilhoai* are presented with comments on karyotypic characteristics of the genus.

Key words: Ancistrini; Heterochromatin; Karyotype evolution; rDNA

INTRODUCTION

Ancistrini Kner, 1854 was resurrected by Amrbruster (2004) as a tribe of the Hypostominae subfamily, and consists of 24 genus and approximately 200 species. Along with Hypostomoni, this tribe has the larger number of species. Among Ancistrini, *Ancistrus* is the most diverse and is composed of 65 valid species (Bifi et al., 2009; Froese and Pauly, 2014). Although the Iguaçu River basin contains relatively few species of fish, it was classified as an important aquatic ecoregion, since 51 to 71% of those species are apparently endemic (Abell et al., 2008). Three recently described *Ancistrus* species are found in the Iguaçu River basin: *Ancistrus abilhoai* (Figure 1), *A. agostinhoi*, and *A. mullerae*. *A. abilhoai* is limited to the upper and middle Iguaçu river portions (Bifi et al., 2009), is classified as an endemic species, and is very rare and hard to find (Baumgartner et al., 2012).

Figure 1. Specimen of *Ancistrus abilhoai* from the Iguaçu River basin. Bar = 20 mm.

In previous cytogenetic papers regarding *Ancistrus*, the 2n ranged from 34 chromosomes in *A. cuiabae* to 54 chromosomes in *A. claro* (Alves et al., 2003; Souza et al., 2004; Alves et al., 2005; Mariotto and Miyazawa, 2006; de Oliveira et al., 2006, 2007, 2008; Mariotto et al., 2004, 2011). Artoni and Bertollo (2001) suggested that a diploid number of 54 chromosomes was the ancestral condition for loricariids. Nevertheless, this number is rare in the Hypostominae subfamily and specifically within Ancistrini, whose members have 2n values ≤ 54 chromosomes, suggesting that centric fusions in particular, contributed to the karyotype evolution of this tribe (Mariotto et al., 2011).

In the present study, cytogenetic data from two populations of *A. abilhoai* from the Iguaçu River basin are presented. The description of the karyotypic structure of a recently described and putative endemic species is an important reference that contributes to our understanding of the chromosomal evolution in this genus.

MATERIAL AND METHODS

Twenty-three *A. abilhoai* individuals from the two populations were analyzed: 11 specimens from the Iguaçu River (26°15'64"S; 51°06'28"W), in the municipality of União da Vitória, Paraná State; and 12 specimens from the Timbó River (26°30'37"S; 50°46'55"W), a tributary of the Iguaçu River, in the municipality of Santa Cruz do Timbó, Santa Catarina State. Voucher specimens were deposited in Coleção Ictiológica do Nupélia - Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Maringá, Paraná, Brazil (NUP-14679). The sex of the animals was determined by gonadal examination.

Chromosomal preparations were obtained from anterior kidney cells following *in vivo* colchicine treatment (Bertollo et al., 1978). Chromosome nomenclature was in accordance with that described by Levan et al. (1964). Constitutive heterochromatin was detected by the C-banding method (Sumner, 1972) as well as by combined staining with 4'-6-diamin-2-phenylindole (DAPI) and chromomycin A_3 (CMA_3) (Schweizer, 1980). Nucleolar organizer regions (NOR) were identified by Ag-NOR staining (Howell and Black, 1980) and by fluorescent *in situ* hybridization (FISH) (Heslop-Harrison et al., 1991). The dual-color FISH technique was performed with an 18S rDNA probe from *Prochilodus argenteus* Spix and Agassiz (Hatanaka and Galetti Jr., 2004) and a 5S rDNA probe from *Leporinus elongatus* Valenciennes (Martins and Galetti Jr., 1999). The probes 18S and 5S were labeled with biotin-14-dATP and digoxigenin-11-dUTP, respectively. Both were labeled by nick translation according to manufacturer instructions (Roche Diagnostics, Basel, Switzerland). The detection and amplification of hybridization signals were carried out using an avidin-FITC conjugate (Sigma-Aldrich, St. Louis, MO, USA) and anti-digoxigenin rhodamine (Roche). FISH signals were analyzed in a Zeiss Axiophot epifluorescence microscope, and the chromosome images were captured by the Case Data Manager Expo 4.0 (Applied Spectral Imaging, MigdalHa'emek, Israel) software.

RESULTS

Both *A. abilhoai* populations exhibited a diploid number of 2n = 48 chromosomes, consisting of 22 metacentric, 14 submetacentric, 6 subtelocentric, and 6 acrocentric chromosomes, with a fundamental number of 90 (Figure 2).

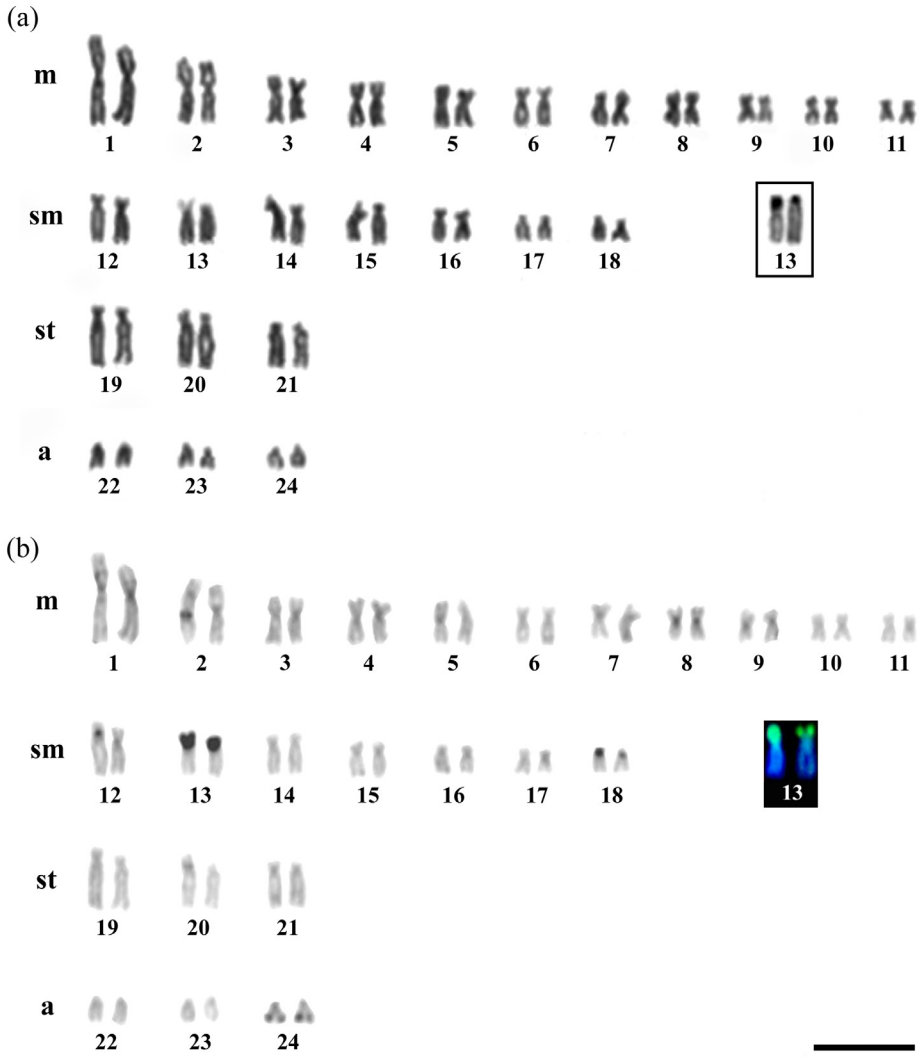

Figure 2. Karyotypes of *Ancistrus abilhoai* arranged from Giemsa-stained (**a**) and C-banding (**b**). The NOR-bearing chromosome pair is shown after silver impregnation (white boxes) and DAPI/CMA3 staining (black boxes). Bar = 10 μm.

The C-banding technique revealed constitutive heterochromatin restricted to the centromeric region on all chromosomes, in the telomeric region of pair 24, and in the extension of the short arm of chromosome pair 13 (Figure 2). This last pair bears 45S rDNA genes as identified by silver nitrate (Ag-NOR) staining and by CMA_3/DAPI fluorochromes (Figures 2 boxes).

Dual-color FISH using 18S and 5S rDNA probes showed adjacent co-localization of these sequences on the short arms of the submetacentric chromosomal pair 13, which is associated with heterochromatic regions (Figure 3).

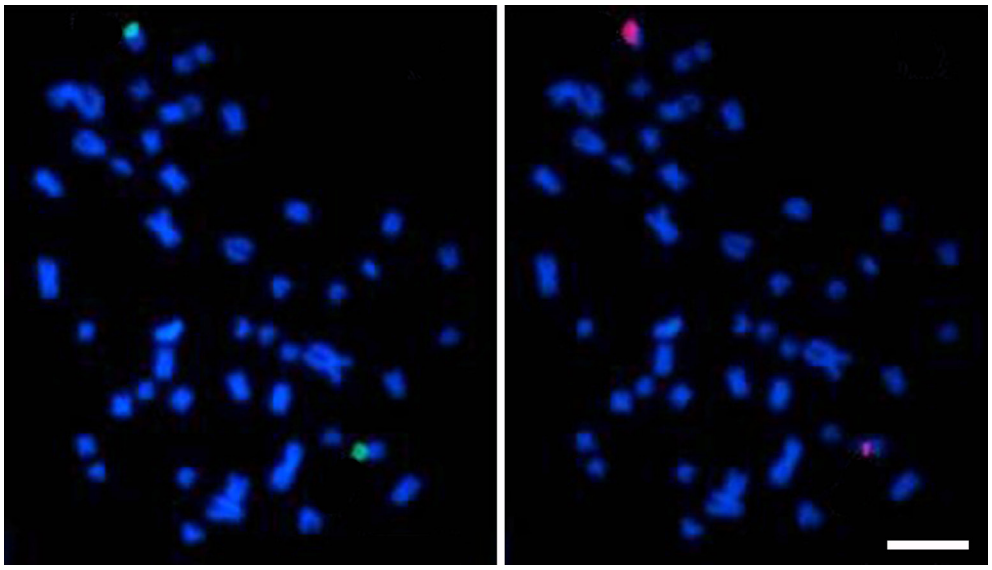

Figure 3. Metaphase of *Ancistrus abilhoai* after double-FISH using 18S rDNA (green signal) and 5S rDNA (red signal) probes. Bar = 10 μm.

DISCUSSION

These data from *Ancistrus* revealed that seven of 27 species have the diploid number of 2n = 52 chromosomes. A karyotype with 48 chromosomes is uncommon in the genus and up to now was only observed in three species, two of them from Iguaçu River basin - *Ancistrus* sp and *A. abilhoai* (analyzed in the present work), and in *A. ranunculus* from the Xingu River, in the Amazonas state. The presence of diploid numbers less than 52 suggests the predominance of Robertsonian chromosomal rearrangements (centric fusion) in the karyotypic evolution of this genus (Alves et al., 2003), although rearrangements such as pericentric inversions and translocations have also had an effect on the chromosomal evolution of this group. These rearrangements could explain the existence of many karyotypes with higher numbers of acrocentric chromosomes (de Oliveira et al., 2009, Mariotto et al., 2011).

The Iguaçu River is an environmentally heterogeneous river with some taxa presenting a relatively high diversity such as the characid *Astyanax* (Garavello and Sampaio, 2010), the siluriform *Trichomycterus* (de Pinna, 1992), and the loricariid *Hypostomus* (Garavello et al., 2012). Due to the high cytogenetic diversity found in *Ancistrus* whether in the Amazon basin (de Oliveira et al., 2009) or in the Paraguay basin (Mariotto et al., 2011), some degree of variability could be present in two allopatric populations of *Ancistrus* in the high heterogeneous Iguaçu River basin. However, no differences were observed among the individuals from the two sites analyzed in this study. This result indicates that populations from the Iguaçu River channel are genetically similar to, and probably have contact with, the population from Timbó River. However, if they are currently isolated from each other, the isolation is a very recent event.

According to our results, the use of FISH techniques and silver-staining, the NORs were found to be adjacent or embedded in C-banded heterochromatin. The association between

constitutive heterochromatin and rDNA cistrons has been frequently reported in Neotropical Actinopterygii (Galetti, 1998). The NORs staining by specific GC fluorochromes is related to the presence of ribosomal RNA genes packaged in heterochromatic structures (Pendás et al., 1993; Artoni and Bertollo, 1999). The synteny of the 18S and 5S ribosomal genes has also been found in other species of *Ancistrus*. Mariotto et al. (2011), suggest that the *A. claro* karyotype with 2n = 54 chromosomes and synteny for rDNA genes could represent the primitive condition for this genus.

Considering the wide diversity of diploid number within *Ancistrus*, the karyotype found in *A. abilhoai*, with 2n = 48 chromosomes, simple NORs and synteny for 5S and 45S rDNA loci, represent a combination of primitive and derived characters. Therefore, integrative studies are important for modern systematic analysis, and cytogenetic approaches might be useful to better clarify our knowledge on the evolutionary history of this fish group.

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