**Ancistrus cf. dubius** (Siluriformes, Ancistrinae), a complex of species. 1. Chromosomal characterization of four populations and occurrence of sexual chromosomes of type XX/XY, in the pantanal basin of Mato Grosso, Brazil

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**Abstract** — Four populations of *Ancistrus cf. dubius* (Loricariidae, Ancistrinae) were analyzed cytogenetically. They were from different rivers and creeks of the Pantanal Basin, in the state of Mato Grosso, Brazil. The populations were from four distinct locations: population A (4F and 8M), Coxipó river, in the county of Chapada dos Guimarães; population B (4F and 11M), Pari Creek, in the county of Cuiabá; population C (18F and 24M), Flechas Creek, the county of Cáceres and population D (15F and 7M), Fundo Creek, in the county of Poconé. The modal diploid number is 2n=42 chromosomes (24M, 10SM and 8ST) and FN=84. Band C shows a system of sexual chromosomes XX/XY in populations B, C and D. The Ag-RONs are simple, with markers on pair 16 of the specimens analyzed. Sexual chromosomes aren’t rare in fishes but *Ancistrus* shows the unique case of two systems of sexual chromosomes, ZZ/ZW and XX/XY, in the specie identified as *Ancistrus cf. dubius*, and specimens of population A has no differentiation of the macro and micro chromosomatic structure between males and females. This suggests an alopatric speciation event of *Ancistrus cf. dubius* and a complex of species thus contributing to the understanding of citotaxonomic-evolutive relations in the Ancistrinae.

**Key words:** Ancistrinae, Ancistrus cf. dubius, sexual chromosomes, Siluriformes, species complex.

**INTRODUCTION**

The presence of sexual chromosomes in fish is relatively small if the diversity of species analyzed in each group is considered. These chromosomes are characterized by the simple systems of XX/XY, ZZ/ZW and multiple sexual chromosomes with heterogametic males and females with or without morphological differentiation (Almeida-Toledo and Foresti 2001). Systems of sexual chromosomes XX/XY appear in various cases and the majority of the species that have this system of sexual chromosomes are Characiformes. Some Siluriformes of the Loricariidae family show a system of sexual chromosomes ZZ/ZW, as described by Andreata et al. (1993) With Hypopomataceae, Scavone and Júlio Jr. (1995) with Lorciarianae and Artost et al. (1998) with Hypostominae; others show sexual chromosomes of the XX/XY type were recorded only by Michelle et al. (1977) with Hypostominae and Andreata et al. (1992) with Hypoptopomataceae. The first appearance of sexual chromosomes in the subfamily Ancistrinae was described by Mariotto et al. (2004), and sexual chromosomes of the type ZZ/ZW were verified in samples identified as Ancistrus cf. dubius. They were collected in small streams of the Serra das Araras (Macaw Mountains) in Mato Grosso, Brazil. These samples presented differences in the macro and micro chromosomic structure, with large blocks of constitutive heterochromatin in the pair 20A, and 2n=44 chromosomes (18M, 10SM, 16ST/A). Was related the second occurrence by de Oliveira et al. (2006) with specimens of Hemiancistrus spilomma, collected in the Araguaia River, Mato Grosso, which presented sexual chromosomes of type ZZ/ZW and multiplicity of Ag-NOR.

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In this study, besides the karyotypic analysis of the species *Ancistrus cf. dubius*, we compare the differences between distinct populations in relation to the sexual chromosomes and distribution of the constitutive heterochromatin. This suggests that there is not just one species but in fact, a complex of species.

**MATERIALS AND METHODS**

Ninety one specimens of *Ancistrus cf. dubius* (Loricariidae, Ancistrinae) were sourced and analyzed, from streams here identified with: population A (4F and 8M), Coxipó river, in the county of Chapada dos Guimarães; population B (4F and 11M), Pari Creek, in the county of Cuiabá; population C (18F and 24M), Flechas Creek, in the county of Cáceres and population D (15F and 7M), Fundo Creek, in the county of Poconé, Basin to the Pantanal, Mato Grosso state, Brazil were cytogenetically studied. The chromosome preparations were obtained by the air drying method that was adapted for fish by Bertollo and others (1978). The constitutive heterochromatin, C-band, was detected according to Sumner (1972) and the Nucleolar Organizer Region (NORs) were detected by impregnation with silver nitrate (Howell and Black 1980). Regions rich in GC bases were identified by tinting with the fluorochrome chromomicina A₃ according to Schweizer (1976). The chromosome were organized in three karyotype groups (Metacentric, Submetacentric and Subtelocentric) and put in descending order of size according to criteria of Levan et al. (1964).

**RESULTS**

The research samples of populations A, B, C and D of *Ancistrus cf. dubius* present the karotype composed of 2n=42 chromosomes with 24 Metacentric, 10 Submetacentric and 8 Subtelocentric chromosomes (figure 1) and the fundamental number FN=84.

![Fig. 1 — Giemsa stained karyotypes of *A. cf. dubius* with 2n=42 chromosomes. a) Male and b) female of population A of Chapada dos Guimarães county, c) male and d) female of population D of Poconé county. Sexual chromosomes, XX/XY, are in the boxes.](image)
The Nucleolar Organizer Regions (Ag-NORs), when submitted to silver nitrate treatment, showed simple marking for all the populations. They were located in the interstitial position of the short arm of pair 16 and this pair presents a secondary constriction, which was submitted to fluorochrome chromomicina A3, the chromosomes showed shiny regions in the same position of chromosomes that were stained by silver nitrate (figure 2). Also observed with CMA3 were shining regions, but with less intensity, in chromosomes of pair 19 in females in one of the chromosomes of this pair in the males of populations B, C and D.

The results obtained by C banding show a variation of distribution of constitutive heterochromatin among the populations A, B, C and D of Ancistrus cf. dubius (figures 3 and 4). Samples of populations B, C and D are similar in distribution of heterochromatic regions, being telomeric and pericentromeric, with the exception of pair 19 that presents an extensive heterochromatic region in the pair 19 of females and in just one of the complements of the pair 19 of males thus characterizing a sexual chromosomal system XX/XY.

In the samples of population A, heterochromatic blocks were verified in the pericentromeric and telomeric regions, for both sexes, without the occurrence of large distinctive blocks of constitutive heterochromatin (figure 3).

**DISCUSSION**

The evident differences of distribution of constitutive heterochromatin between males and females characterized by sexual chromosomes of type XX/XY in populations B, C and D, where the male represent the heterogametic sex. The XX pair of chromosomes shows a large heterochromatic block which almost occupies the entire long
arm of pair 19, Subtelocentric, in females while in the males only one chromosome of the pair is heterochromatic. Chromosome Y has a euchromatic morphological structure.

The process of heterochromatination has been associated with evolution of sexual chromosomes in diverse groups of fish (Galetti and Foresti 1986; Bertollo and Cavallaro 1992; Moreira-Filho et al. 1993). The differentiations of sexual chromosomes can occur by accumulation or loss of heterochromatin (Almeida-Toledo and Foresti 2001). In the present work the hypothesis is that occurred due to loss of regions rich in GC in one of the complements of a pair of chromosomes, after the sexual differentiation, which in this case is the chromosome Y. This evolutionary process also must have occurred with the chromosome W in Ancistrus cf. dubius with 2n=44 and the sexual chromosome system ZZ/ZW (Mariotto et al. 2004), besides the Robertsonian mechanisms that must be considered in the species forming processes.

The population A of the county of Chapada dos Guimarães didn’t show differences in distribution of constitutive heterochromatin between males and females, however, each sexual chromosome is perhaps due to the location or the small number of individuals analyzed. A larger sample could clarify the differences and similarities of this population in relation to the others. In any case, the samples showed similar Ag-NORs, diploid number and karyotypic structure in relation to the other populations studied.

Previous studies have shown that in some cases there are two systems of sexual chromosomes in the same genus as in Eigenmania (Almeida-Toledo et al. 1984), Hoplias (Bertollo et al. 2000) and in the same subfamily but in different genus as in the case of the species Microlepidogaster leucofrenatus (Andreatta et al. 1994) and Pseudocinclus tietensis (Andreatta et al. 1992) of the subfamily Hypoptopomatinae. In these two species the heterochromatic chromosome is the W or Y. This suggests that the evolu-
tionary mechanisms for the differentiation of sexual chromosomes are variable from one group of fish to another. However, heterochromatin analyses are scarce among the Siluriformes, despite the fact that its distribution and composition can give important insights to karyotypic evolution (Kavalco et al. 2004).

The low vagility shown by Ancistrinae can represent an important factor for explaining how close populations can show distinct karyotypes as found in the samples of this study, and compared to those found in the work of Mariotto et al. (2004). At the moment some Ancistrinae species appear to retain probable ancestral features of the Loricariidae, as lower chromosomal number and fundamental diploid number (Lara and Júlio-Jr. 1994; Artoni and Bertollo 2001). Also evident are the diversity of diploid number (2n=38 to 2n=52) and fundamental number that shows karyotypic evolution in the Ancistrinae (Alves et al. 2003).

Due to variation shown in the samples analyzed of Ancistrus cf. dubius, with 2n=44 and sexual chromosomes of the type ZZ/ZW (Mariotto et al. 2004) and Ancistrus cf. dubius with 2n=42 with sexual chromosomes type XX/XY or without sexual chromosomes, where there are evident differences shown in the same species, which is unique at the moment, and being that these population are isolated probably due to the low vagility presented by the group, we can hypothesize that there are three distinct cytotypes, constituting a complex of species for Ancistrus cf. dubius, one with 2n=44 and ZZ/ZW, another with 2n=42 and XX/XY and another without sexual chromosomes.

Cytogenetic studies must to be increased in this subfamily to elucidate its chromosomal evolutionary history (Artoni and Bertollo 2001). Other techniques too can contribute to clarify the evolutive history of the Loricariidae family and to assist in defining the phylogeny of neotropical fish. Even though there are no evident morphological differences these results, indicate the necessity of a better systematic revision of this group and the importance of new studies.

Fig. 4 — a and b) Band C of male and female of population C, with sexual chromosomes type XX/XY. c and b) Band C of male and female of population D, showing pair 19 with XX/XY.
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REFERENCES


