

Occurrence of multiple sexual chromosomes (XX/XY_1Y_2 and $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$) in catfishes of the genus *Ancistrus* (Siluriformes: Loricariidae) from the Amazon basin

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Abstract Loricariid catfishes show a predominance of homomorphism in sex chromosomes, but cases of simple and multiple systems were also found. Here we describe two cases of multiple sex chromosome systems in loricariids from Brazilian Amazonia. Males of *Ancistrus* sp.1 “Balbina” have a modal number of $2n = 39$ chromosomes, fundamental number (FN) of 78, and karyotypic formula of $27 m + 10 sm + 2 st$; females have $2n = 38$ chromosomes, FN = 76, and $26 m + 10 sm + 2 st$. *Ancistrus* sp.2 “Barcelos” has $2n = 52$ chromosomes for both sexes, FN = 80 for males and FN = 79 for females. Karyotypic formula is $12 m + 12 sm + 4 st + 24a$ for males and $11 m + 12 sm + 4st + 25a$ for females. The two species show different arrangements of constitutive heterochromatin blocks, which are coincident with NORs and absent in sex chromosomes. We suggest a XX/XY_1Y_2 mechanism for *Ancistrus* sp.1 “Balbina”, and a $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$ mechanism for *Ancistrus* sp.2 “Barcelos”. The XX/XY_1Y_2 mechanism here reported is the second known occurrence of this type of multiple sex chromosomes for Loricariidae and the third for Neotropical fishes; the mechanism $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$ represents the first record among fishes. The presence of different sex chromosome systems in *Ancistrus* indicates a probable independent origin and suggests that the differentiation of sex chromosomes is evolutionarily recent among species in this genus.

Keywords Ancistrini · Cytogenetics · Evolution · Karyotype · Neotropical fishes

Introduction

The majority of Neotropical fish species that have been studied cytogenetically do not have differentiated sex chromosomes (Moreira-Filho et al. 1993; Centofante et al. 2002). However, a variety of differentiated sex chromosome systems have been described for this ichthyofauna (e.g. Galetti et al. 1981; Bertollo et al. 1997; Bertollo and Mestriner 1998; Almeida-Toledo et al. 2001; Centofante et al. 2002; Venere et al. 2004; Alves et al. 2006; de Oliveira et al. 2006). These systems may involve only one pair of originally homologous chromosomes (XX/XY , XX/XO and ZZ/ZW), referred to as simple systems, or several of the chromosomes of the complement ($X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2 and ZZ/ZW_1W_2), called multiple systems.

XX/XY systems have been documented in species of Gymnotiformes, *Eigenmannia virescens* (Almeida-Toledo et al. 2001), Cyprinodontiformes, *Poecilia reticulata* (Nanda et al. 1990), and Characiformes, *Hoplias malabaricus* (Born and Bertollo 2000). ZZ/ZW systems are more frequent and occur in a diversity of families, such as Anostomidae (Galetti et al. 1981; Venere et al. 2004), Prochilodontidae (Feldberg et al. 1987), Characidae (Bertollo and Cavallaro 1992; Maistro et al. 1998) and Parodontidae (Moreira-Filho et al. 1993; Centofante et al. 2002) in Characiformes, and Poeciliidae in Cyprinodontiformes (Haaf and Schmid 1984). Multiple systems have been documented in Characiformes of the families Parodontidae (Moreira-Filho et al. 1980) and Erythrinidae (Bertollo et al. 1983; Dergam and Bertollo 1990; Bertollo et al. 1997; Bertollo and Mestriner 1998) and in

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Gymnotiformes of the families Sternopygidae (Almeida-Toledo et al. 1984) and Hypopomidae (Almeida-Toledo et al. 2000).

Although cytogenetic studies of the family Loricariidae have revealed the predominance of homomorphic sex chromosomes (Artoni and Bertollo 2001; Alves et al. 2005; Kavalco et al. 2005), cases of simple and multiple systems of sex chromosomes have been described for this group. In the subfamily Hypostominae, XX/XY (Michelle et al. 1977; Mariotto and Miyazawa 2006), XX/XO (Alves et al. 2006), and ZZ/ZW (Artoni et al. 1998; Mariotto et al. 2004; de Oliveira et al. 2006) sex chromosome systems have been found. XX/XY (Andreato et al. 1992) and ZZ/ZW (Andreato et al. 1993; 1994) systems have been described for species of Hypoptopomatinae, and ZZ/ZW (Scavone and Júlio Jr. 1995) and XX/XY₁Y₂ (Centofante et al. 2006) systems have been described for Loricariinae.

Here we describe two cases of multiple sex chromosome systems found in two species of loricariids of the genus *Ancistrus* from the Brazilian Amazonia.

Material and methods

All specimens were collected from small streams in the state of Amazonas, Brazil. Since the species studied here are most likely new to science (S. Fish-Muller, pers. comm.), we present a short description of color pattern and include images of a preserved specimen for each species. Voucher specimens were deposited in the fish collection at the National Institute for Amazonian Research (INPA).

Ancistrus sp.1 “Balbina” (INPA 25633): 12 specimens (six males, six females) collected in the Barretinho Stream, an affluent of the Uatumã River in the municipality of Presidente Figueiredo, approximately 180 km north of

Manaus (S 01°58'20", W 59°29'48"). Specimens have a brown dorsal region with olive spots a little larger than the pupilla; ventral region dark with large, well-defined light olive spots; dorsal fin with diffuse, alternating black and white stripes (Fig. 1a).

Ancistrus sp.2 “Barcelos” (INPA 25627): 11 specimens (seven males, four females) collected from the Demeni River, middle portion of Negro River basin, in the municipality of Barcelos (S 00°25'19", W 062°54'42"). This species has a black dorsal region finely stippled with tiny white spots; ventral region dark with well-defined pale spots, larger than those on dorsal region; dorsal fin black with white stipples and a white border (Fig. 1b).

Chromosomal preparations were obtained from kidney cells, following the “air-drying” technique of Bertollo et al. (1978). The constitutive heterochromatin was identified according to Sumner (1972) and the nucleolar organizing regions (NORs) were detected using the technique described by Howell and Black (1980). Chromosomes were classified based on arm ratios as metacentrics (m), submetacentrics (sm), subtelocentrics (st), and acrocentrics (a) as proposed by Levan et al. (1964). The fundamental number (FN) or arm number was determined by considering meta-, submeta- and subtelocentric chromosomes with two arms and acrocentrics with only one.

Results

Males of *Ancistrus* sp.1 “Balbina” have a modal number of $2n = 39$ chromosomes, fundamental number FN = 78, and karyotypic formula of $27 m + 10 sm + 2 st$; females have a modal number of $2n = 38$ chromosomes, FN = 76, and karyotypic formula of $26 m + 10 sm + 2 st$ (Fig. 2a and b). NORs are located in the terminal region of the long arms of pair 12 (Fig. 2c).

Fig. 1 Lateral, dorsal and ventral views of *Ancistrus* sp.1 “Balbina” (INPA 25633), 83 mm SL (a), and *Ancistrus* sp.2 “Barcelos” (INPA 25627), 92 mm SL (b)

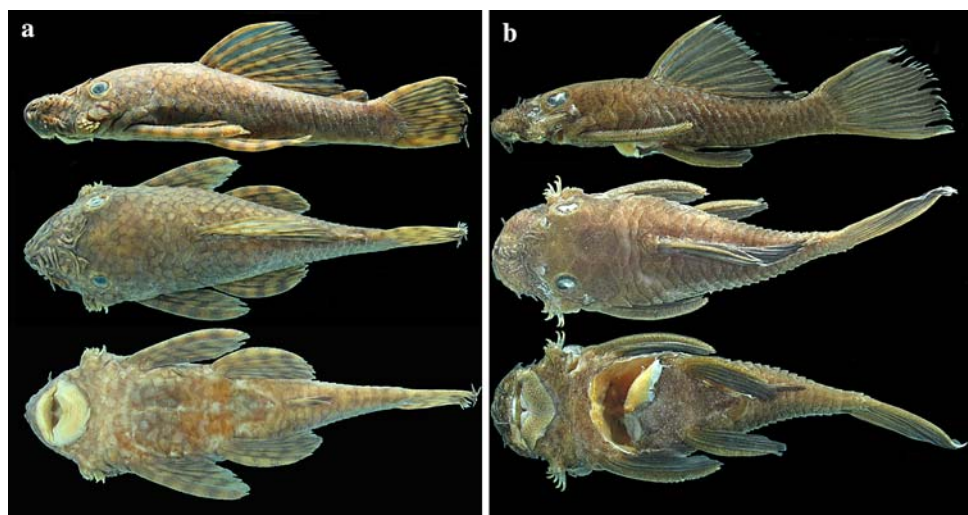
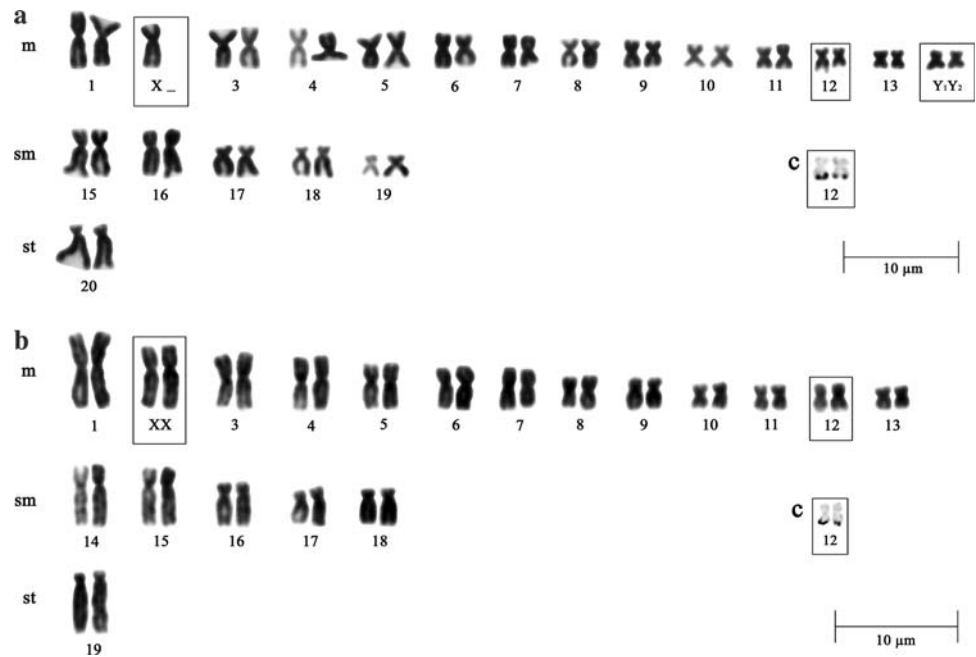


Fig. 2 Karyotypes of male (a) and female (b) *Ancistrus* sp.1 “Balbina” after conventional Giemsa-staining, evidencing the sex chromosomes (box) and the NOR-bearing chromosomes after silver-staining (c). m = metacentric; sm = submetacentric; st = subtelocentric



Ancistrus sp.2 “Barcelos” has a modal number of $2n = 52$ chromosomes in both sexes and $FN = 80$ for males and $FN = 79$ for females. The karyotypic formula is $12m + 12sm + 4st + 24a$ for males, and $11m + 12sm + 4st + 25a$ for females (Fig. 3a and b). Heteromorphic NORs are found in the short arms of pair 23 (Fig. 3c).

Ancistrus sp.1 “Balbina” has interstitial blocks of constitutive heterochromatin in the short arms of pairs 3, 4, and 14, and in the long arms of pair 6; pericentromeric blocks in pairs 8 and 9; distal blocks in the long arms of pair 12; and proximal blocks in long arms of pair 19 (Fig. 4a). *Ancistrus* sp.2 “Barcelos” has small pericentromeric blocks in pairs 15, 16, and 17; conspicuous blocks in the short arms of pairs 14 and 23, in an interstitial position in the long arms of pairs 1 and 11, and in the short arms of pair 2 (Fig. 4b). In both species, NORs are coincident with heterochromatin blocks, which are absent in sex chromosomes (Fig. 4a and b).

Discussion

Moreira-Filho et al. (1993) summarized five sex chromosome systems that have been described for Neotropical freshwater fishes: ZZ/ZW, XX/XY, $X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2 , and ZZ/ZW₁W₂. Most recently, Alves et al. (2006) described a sixth system, XX/XO. Thus far, 63 occurrences of heteromorphic sex chromosomes have been described for this group of fish. Sixty-three and a half percent of these represent cases in which females are heterogametic, 36.5% in which males are heterogametic, and 19% are cases of multiple systems (Centofante et al. 2002; Mariotto et al. 2004; Venere et al. 2004; Alves et al. 2006;

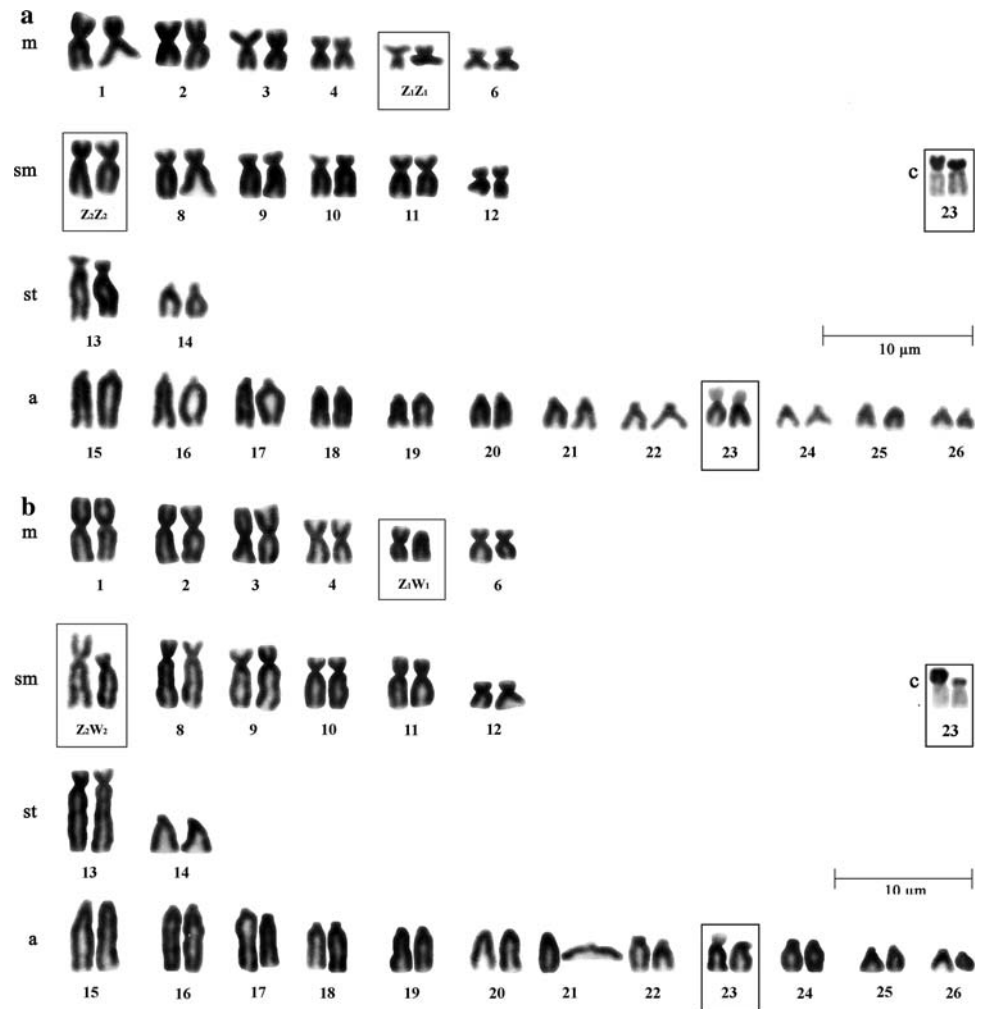
Centofante et al. 2006; de Oliveira et al. 2006; Mariotto and Miyazawa 2006).

In the genus *Ancistrus*, as in Loricariids in general, few cases of heteromorphic sex chromosomes have been described, all of them representing simple sex determination systems. ZZ/ZW and XX/XY systems were found in distinct populations of *Ancistrus* cf. *dubius* of the Brazilian wetlands of the Pantanal in Mato Grosso State (Mariotto et al. 2004; Mariotto and Miyazawa 2006). The only XX/XO system ever documented in a Neotropical fish was found in *Ancistrus* n. sp.1 of the Vermelho River in Goiás State, Brazil (Alves et al. 2006).

We found two types of multiple sex chromosome mechanisms in *Ancistrus* species from the central Amazonia. We suggest a XX/XY_1Y_2 mechanism for *Ancistrus* sp.1 “Balbina”, based on the presence of an additional chromosome in the male karyotype. In this species, the X chromosomes are represented by large metacentric chromosomes (pair 2 of the complement) and Y chromosomes by small metacentrics (pair 14 of the male complement). We propose a $Z_1Z_1Z_2Z_2/Z_1Z_2W_1W_2$ mechanism for *Ancistrus* sp.2 “Barcelos”, based on heteromorphism in the female karyotype: chromosome Z_1 is a small metacentric (pair 5 of the complement), chromosome Z_2 is the largest submetacentric of the complement (pair 7), W_1 is a small acrocentric, and W_2 is a medium-sized submetacentric.

The multiple mechanism of the XX/XY_1Y_2 type found in *Ancistrus* sp.1 “Balbina” represents the second documented occurrence of this type of multiple sex chromosomes for Loricariidae, and the third for Neotropical fishes overall. This type of system has been described

Fig. 3 Karyotypes of male (a) and female (b) *Ancistrus* sp.2 “Barcelos” after conventional Giemsa-staining, evidencing the sex chromosomes (box) and the NOR-bearing chromosomes after silver-staining (c). m = metacentric; sm = submetacentric; st = subtelo-centric; a = acrocentric



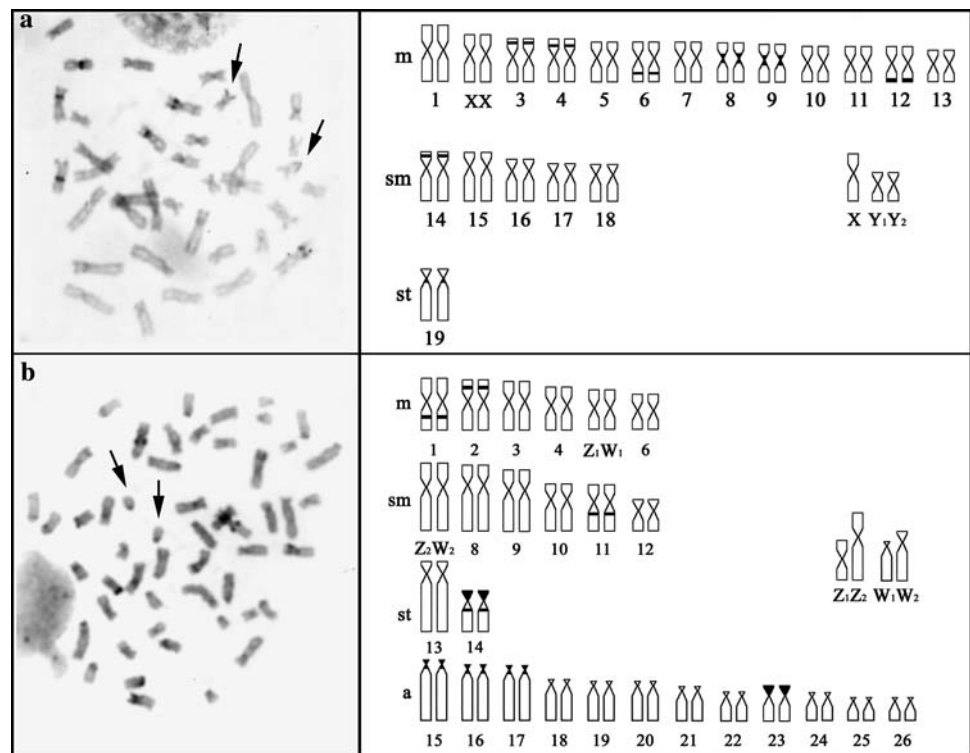
for the erythrinid *Hoplias malabaricus* (Bertollo et al. 1983) and for the loricariid *Harttia carvalhoi* (Centofante et al. 2006). The ZZ/ZW₁W₂ multiple mechanism, in which the female is the heterogametic sex, has previously been described only in the parodontid *Apareiodon affinis* (Moreira-Filho et al. 1980). The occurrence of centric fissions seems to be the most plausible explanation for the origin of these sexual systems (Moreira-Filho et al. 1980; Bertollo et al. 1983). However, Centofante et al. (2006) believe that, in *Harttia carvalhoi*, sex chromosomes have been originated by centric fusions or translocations, since the other species in the genus show higher diploid numbers. Furthermore, karyotypes of these species show that all chromosome pairs are relatively the same size, whereas the first pair of chromosomes in *H. carvalhoi* is much larger than the other pairs in the complement.

Alves et al. (2003) suggested that centric fusion predominate among the rearrangements that occurred in the evolution of karyotypes of *Ancistrus* species. Therefore it is likely that centric fusions, followed by pericentric inversions in the chromosomes Y₁Y₂, are responsible for the

differentiation of sex chromosomes in *Ancistrus* sp.1 “Balbina”. This species has a lower diploid number compared to the supposedly basal condition among the Ancistrini (namely, 2n = 52 chromosomes, the majority meta/submetacentric; Artoni and Bertollo 2001; Alves et al. 2003). We can hypothesize two pathways for the origin of the karyotype observed in males of *Ancistrus* sp.1 “Balbina”: (1) fusion of a set of acrocentric chromosomes, followed by centric fission of one of the homologues of pair 2, generating two small acrocentrics; subsequent pericentric inversions in these chromosomes would have generated the Y₁Y₂ small metacentrics; or (2) the initial chromosome fusion event would have involved just one of the homologues of pair 2; then translocations in the two remaining acrocentrics would have generated the Y₁Y₂ small metacentrics. In females, the complete fusion of the corresponding set of acrocentrics would have generated the X chromosomes and the karyotype of 2n = 38.

The multiple sex chromosome mechanism Z₁Z₁Z₂Z₂/Z₁Z₂W₁W₂ we propose for *Ancistrus* sp.2 “Barcelos” is the first observation of this type of system in fish. A similar

Fig. 4 Metaphase and Idiogram showing the distribution of constitutive heterochromatin after C-banding technique in *Ancistrus* sp.1 “Balbina” (a) and in *Ancistrus* sp.2 “Barcelos” (b). Arrows indicate the NOR-bearing chromosomes with positive C-banding. m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric



mechanism was found in a species of flea (*Nosopsyllus fasciatus*; Aphaniptera), but where the male is the heterogametic sex (Bayreuther 1969). Since the karyotype of *Ancistrus* sp.2 “Barcelos” is made up of $2n = 52$ chromosomes and about half its complement consists of acrocentric chromosomes, we suggest that pericentromeric inversions and translocations have been involved in both the karyotypic evolution as well as the differentiation of sex chromosomes in this species.

The most common mechanism involved in the differentiation of simple sex chromosome systems is the accumulation of heterochromatin. However, structural events are also important and are often associated with sequences of repetitive DNA, as proposed by Artoni et al. (2001) and Artoni and Bertollo (2002) for differentiation of the W chromosome in some *Triporthus* species (Characiformes: Characidae). Multiple sex chromosome systems are believed to originate from pre-existing simple systems, arising mainly through rearrangements involving sex and autosomic chromosomes (Guerra 1988). The rearrangements most commonly proposed are: (1) translocations, such as those found in populations of *Hoplias malabaricus* (Bertollo et al. 1983; Bertollo et al. 1997); (2) centric fusion or Robertsonian translocation, as in *Eigenmannia* sp. 2 (Almeida-Toledo et al. 1984) and *Brachyhypopomus pinnicaudatus* (Almeida-Toledo et al. 2000); and (3) centric fissions, like those observed in *Apareiodon affinis* (Moreira-Filho et al. 1980). The absence of a heterochromatin accumulation in the differentiation of

multiple chromosome systems in *Ancistrus* supports our hypothesis of an origin through a simple system.

Congeneric species and even different populations of the same species can exhibit different sex chromosome systems and vary in which sex is heterogametic, which point out to different stages of sex chromosome differentiation (Almeida-Toledo and Foresti 2001; Mariotto et al. 2004; Mariotto and Miyazawa 2006). Therefore, the presence of different sex chromosome systems in species of *Ancistrus* indicates a probable independent origin of these systems, and suggests that the differentiation of sex chromosomes is evolutionarily recent among species in this genus.

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