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A novel ZZ/ZW sex chromosome system for the genus *Leporinus* (Pisces, Anostomidae, Characiformes)

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Abstract

A wide range of sex chromosome mechanisms, including simple and multiple chromosome systems is characteristic of fishes. The *Leporinus* genus represent a good model to study sex chromosome mechanisms, because an unambiguous ZZ/ZW sex chromosome system was previously described for seven species, while the remaining studied species of the genus do not show differentiated sex chromosomes. The occurrence of sex chromosomes in *Leporinus trifasciatus* and *Leporinus* sp2 from the Araguaia river, Amazon basin, Brazil, was here investigated. ZZ/ZW sex chromosomes were detected for both species. The Z and W chromosome morphology of *L. trifasciatus* is the same as described for other species of the genus *Leporinus*. However, the Z and W chromosomes of *L.* sp2 were quite different in their morphology and banding pattern suggesting that the ZW system of this species have originated independently from the ZW system previously described for other *Leporinus*.

Introduction

Cytological advances over the last few years have improved the information on fish chromosomes. Considering sex differentiation, fish are characterized by remarkable variability of sex-determination mechanisms, including polyfactorial systems, male and female heterogamety, hermaphroditism, and environmental sex determination (Yamamoto, 1969; Conover, Van Voorhes & Ehtisham, 1992). While XX/XY and ZZ/ZW sex chromosome systems are characteristic for mammals and birds, respectively, eight types of sex chromosome systems have already been described for fishes (Moreira-Filho, Bertollo & Galetti, 1993; Almeida Toledo & Foresti, 2001).

Extensive karyotype diversity has been reported in the neotropical fish fauna and several studies have demonstrated the occurrence of sex chromosomes in different groups, especially in freshwater species. Different male or female heterogamety can be found among related species (Centofante, Bertollo & Moreira-Filho, 2002). A XX/XY sex chromosome system has been described for four neotropical freshwater fish species, representing four different orders (Almeida Toledo & Foresti, 2001). On the other hand, a ZZ/ZW system has been described in several families of: (i) Characiformes, such as Anostomidae (Galetti et al., 1981b; Galetti, Lima & Venere, 1995), Characidae (Bertollo & Cavallaro, 1992), Prochilodontidae (Feldberg et al., 1987) and Parodontidae (Moreira Filho, Bertollo & Galetti, 1993); (ii) Siluriformes, such as Loricariidae (Andreatta et al., 1993; Artoni, Venere & Bertollo, 1998); (iii) Cyprinodontiformes, such as Poecilidae (Haaf & Schmid, 1984). Also, three different multiple sex chromosome mechanisms occur for several species of neotropical fish fauna, including $X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2 and ZZ/ZW_1W_2 systems (Centofante, Bertollo & Moreira-Filho, 2002). Although several sex chromosome systems appear in fish, most species do not show differentiated sex chromosomes. The diversity of mechanisms that appears in fish, even within some

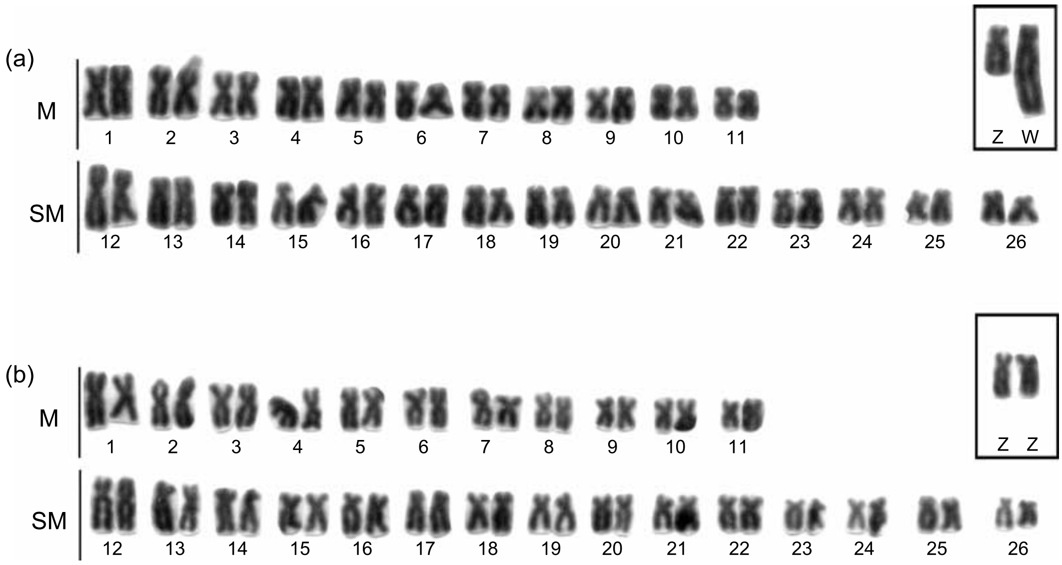

Figure 1. Giemsa karyotypes of female (a) and male (b) of *L. trifasciatus*. The sex chromosomes are in the inset. Secondary constriction can be visualized on the short arm of pair 2.

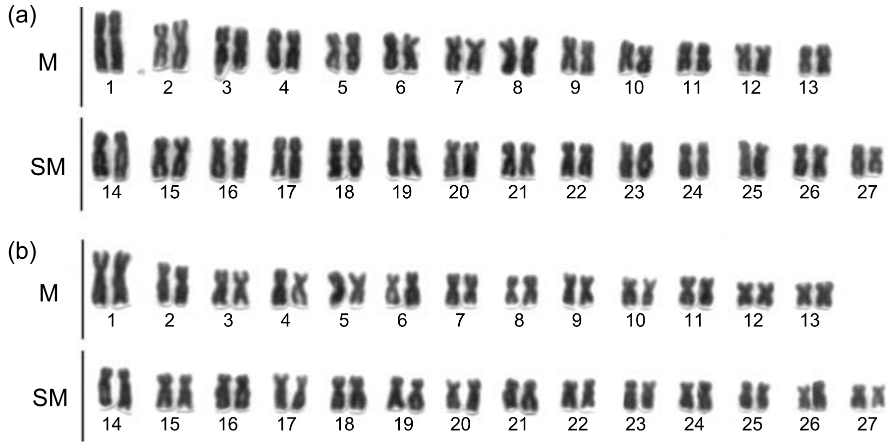

Figure 2. Giemsa karyotypes of female (a) and male (b) of *Leporinus* sp2. The Z and W chromosomes (pair 1) are morphologically undistinguishable in the female Giemsa karyotype. Secondary constriction can be observed on the long arm of pair 3.

individual species, and also the absence of sex chromosomes in most of them, indicate that this class of animals provides a rich source of material for studying the evolutionary process of sex determination origin in vertebrates.

The *Leporinus* genus (Anostomidae, Characiformes) represents a good model to study sex determination mechanisms. A clear ZZ/ZW sex chromosome system has been described for seven species (*L. conirostris*, *L. trifasciatus*, *L. obtusidens*, *L. elongatus*, *L. macrocephalus*, *L. reinhardti* and *L.* aff *elongatus*), while the remaining species of the genus that have been cytogenetically studied (around 40 species) have no differentiated sex chromosomes (Galetti, Lima & Venere, 1995). In the present paper, the karyotypes of two freshwater *Leporinus* fish species – *L. trifasciatus* and *L.* sp2 (sensu Santos & Jegu, 1989) – were studied, and a novel ZZ/ZW sex chromosome mechanism, morphologically differentiated from the ZZ/ZW system previously detected, is described for *L.* sp2.

Materials and methods

Mitotic chromosomes of two *Leporinus* species were analyzed: *L. trifasciatus* (1 female and 3 males) and

L. sp2 (13 females and 7 males). All species were collected in the Araguaia River (Amazon basin), townships of Barra do Garças-MT and Aragarças-GO, Brazil.

Chromosome preparations were obtained from cell suspension of the cephalic portion of the kidney by standard methods, basically as described by Bertollo, Takahashi & Moreira-Filho (1978). The nucleolus organizer regions (NORs) were visualized by the silver staining (Ag-NOR) method (Howell & Black, 1980), the constitutive heterochromatin was identified with barium hidroxide according to Sumner (1972) and the fluorochrome mithramycin (MM) staining was obtained following the protocol described in Schmid (1980).

Results

The two *Leporinus* species analyzed were characterized by the presence of 54 biarmed chromosomes in both sexes (Figures 1 and 2). *L. trifasciatus* exhibited a ZZ/ZW sex chromosome system. The Z and W morphology of *L. trifasciatus* was similar to the Z and W described for the other *Leporinus* species and for the population of *L. trifasciatus* from Madeira River (Amazon basin) (Galetti, Lima & Venere, 1995), where the Z is a small submetacentric and the W a large subtelocentric chromosome (Figure 1). *L.* sp2 also presented a ZZ/ZW sex chromosome system, but the Z and W chromosome morphology was quite different from the Z and W detected in the other seven *Leporinus*, including *L. trifasciatus* (Figure 2). The Z and W of *L.* sp2 are both metacentric and correspond to the largest chromosome pair of the karyotype (pair 1 of Figure 2), but the W short arm is longer than the short arm of the Z chromosome (Figure 2).

The C-banding technique detected weak heterochromatic blocks in the centromeres and telomeres of almost all chromosomes of the two studied species (data not shown). The Z chromosome of *L. trifasciatus* exhibited a large heterochromatic block in the long arm, and the W had the long arm fully heterochromatic (Figure 3). The fluorochrome MM staining showed that the heterochromatin of the Z and W chromosomes is heterogeneous, with positive and negative MM stained segments (Figure 3(a)). The difference between the Z and W chromosome of *L.* sp2 is related to the presence of a heterochromatic block in the short arm of the W chromosome that is absent in the Z chromosome (Figures 3(b) and 4(a) and (b)). The chromosomes of *L.* sp2 exhibit a homogeneous staining under the fluorochrome MM treatment, with exception of the heterochromatic block of the W chromosome that was positively stained by this fluorochrome (Figures 3(b) and 4(c) and (d)).

The Ag-NOR sites were detected in the terminal position of a metacentric chromosome pair of the two analyzed species (data not shown), coinciding with secondary constrictions on the short arm of pair 2 in *L. trifasciatus* (Figure 1) and on the long arm of pair 3 in *L.* sp2 (Figure 2), and were not related to the sex chromosomes.

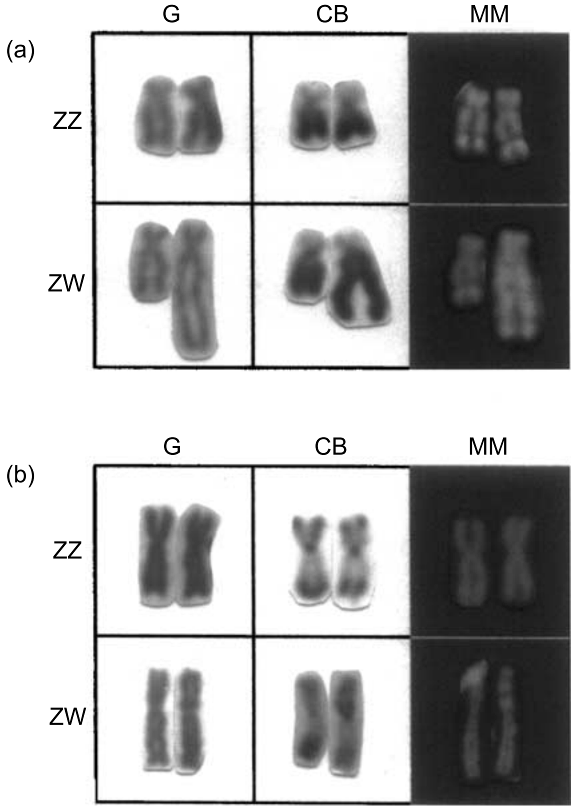

Figure 3. ZZ/ZW sex chromosomes of *L. trifasciatus* (a) and *Leporinus* sp2 (b) under Giemsa staining (G), C-banding (CB) and mithramycin staining (MM). In *L. trifasciatus*, a brighter MM positive band can be visualized at the end of the C-banded heterochromatic segment on the long arm of Z chromosome, while at least three interspaced MM positive bands can be distinguish along the C-banded heterochromatic segment on the long arm of W chromosome. In *L.* sp2, an additional C-banded heterochromatic segment can be observed extending from centromeric region only on the short arm of W chromosome, also revealed by a MM positive band.

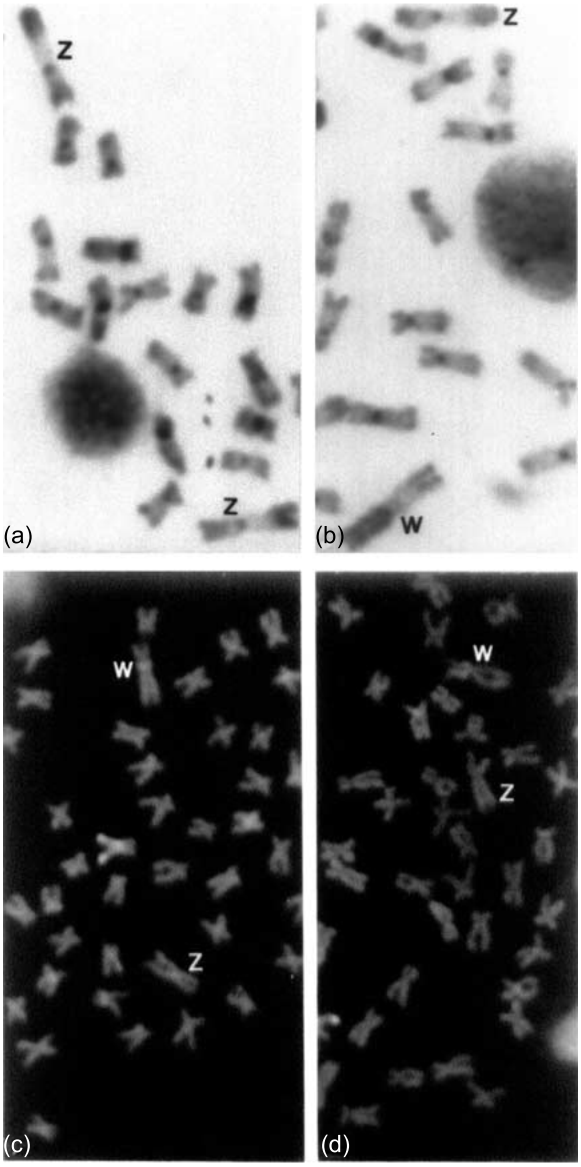

Figure 4. Partial metaphases of *Leporinus* sp2 reinforcing the occurrence of a sexual chromosome system in male (a) and female (b–d). a and b are partial C-banded metaphases showing the additional heterochromatic block in the W chromosome. c and d are partial mithramycin stained metaphases showing a MM positive band on the short arm of W chromosome. Z and W chromosomes are indicated.

Discussion

Although heteromorphic sex chromosomes are absent in most species, new occurrences of sex chromosome systems are often described among different fish groups of the Neotropical fauna. To date, differentiated sex chromosomes have been described for 32 species, which represent 4% of the total number of species already cytogenetically analyzed (Almeida Toledo & Foresti, 2001). A very interesting feature of the sex chromosomes in the neotropical freshwater fauna is the occurrence of a wide range of sex chromosome systems, involving simple XX–XY and ZZ–ZW, and multiple X_1X_2Y, XY_1Y_2 and ZW_1W_2 systems.

Previous cytogenetic data described for the anostomids genera *Leporinus*, *Leporellus*, *Schizodon* (Galetti et al., 1981a), *Abramites*, *Anostomus* and *Pseudanus* (Martins et al., 2000), have shown a common karyotype pattern with $2n = 54$ M-SM chromosomes, confirming the hypothesis that 54 bi-armed chromosomes should be ancient in the family Anostomidae (Galetti et al., 1981a). Variations of the M-SM karyotype pattern among this fish group were mainly related to a peculiar ZZ/ZW sex chromosome system in *Leporinus* species (Galetti et al., 1981b; Galetti & Foresti, 1986; Galetti, Lima & Venere, 1995; Molina, Schmid & Galetti, 1998) and the presence of supernumerary chromosomes in *Schizodon nasutus* and *Leporinus* sp (Pastori, Fenocchio & López, 1997; Venere, Miyazawa & Galetti, 1999). While the sex chromosomes of the *Leporinus* species are highly differentiated, the remaining chromosomes of the complement are included in the typical M-SM pattern.

Although a ZW sex chromosome system has been described for several species of four related families of Characiformes, that is, Anostomidae (Galetti et al., 1981b; Galetti & Foresti, 1986; Galetti, Lima & Venere, 1995), Prochilodontidae (Feldberg et al., 1987), Parodontidae (Moreira-Filho, Bertollo & Galetti, 1993) and Curimatidae (M.C. Navarrete, personal communication), no relation of this system among the different families have been proposed.

Among 44 anostomid species so far studied, only seven *Leporinus* species have cytological differentiated sex chromosomes (Galetti et al., 1981b; Galetti & Foresti, 1986; Galetti, Lima & Venere, 1995; Molina, Schmid & Galetti, 1998). These species show a ZZ/ZW system, in which a large and almost fully heterochromatic subtelocentric is a typical W chromosome, only present in the female karyotype. In contrast, the Z chromosome presents in both sexes is heterochromatic on the distal third part of the long arm. An initial heterochromatinization could be the first step in the differentiation of these sex chromosomes (Galetti & Foresti, 1986). The morphological similarity of chromosomes Z and W among all ZW

Leporinus suggests a common origin for these chromosomes (Galetti, Lima & Venere, 1995).

The occurrence of ZZ/ZW sex chromosomes in *L. trifasciatus* was previously described for the population of Madeira river (Porto Velho, RO, Brazil) (Galetti, Lima & Venere, 1995), and the present results show that this chromosome system is also present in the Araguaia river population. The ZZ/ZW sex chromosomes detected in *Leporinus* sp2 are morphologically different from the ZZ/ZW system previously described for the seven *Leporinus* species. The cytogenetic analysis, so far, was not able to identify similarities between both Z and W chromosomes of *Leporinus* sp2 and the Z and W chromosomes of the other *Leporinus*, respectively, suggesting the occurrence of a novel ZZ/ZW chromosome system in the genus *Leporinus*. The heterochromatic MM positive block, that represents the difference between the Z and W chromosomes of *L.* sp2, seems to have arisen by a gain of a new heterochromatin in the W chromosome. Although there are other heterochromatins in the centromeric and terminal position of the Z chromosome, these segments are not MM positive. The MM positive nature of the heterochromatic block of the W, strongly suggest the presence of a different type of chromatin in this region, without homology in the Z chromosome.

Even among related fishes, such as the *Leporinus* genus, different sex chromosome systems seem to have arisen. Studies in the molecular biology field, isolating DNA sequences of the Z and W chromosomes, represent a promising step in the comprehension of origin and evolution of sex chromosomes in the genus *Leporinus* and, consequently in fish and vertebrates.

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