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An unusual ZZ/ZW sex chromosome system in *Characidium* fishes (Crenuchidae, Characiformes) with the presence of rDNA sites

R. B. Noleto*†, A. P. Amorim*, M. R. Vicari‡, R. F. Artoni‡ and M. M. Cestari*

*Universidade Federal do Paraná, Departamento de Genética, Centro Politécnico, Caixa Postal 19071, CEP 81531-990, Curitiba, PR, Brazil and ‡Universidade Estadual de Ponta Grossa, Departamento de Biologia Estrutural, Molecular e Genética, Ponta Grossa, PR, Brazil

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Characidium fishes with a sex chromosome system form a monophyletic group. This work presents data of *Characidium lanei* from the South Atlantic basin (Brazil), including an unknown type of ZW sex chromosome system for the groups including the presence of rDNA sites on sex chromosomes.

Key words: *Characidium* fish; cytotaxonomy; rDNA; sex chromosomes.

Crenuchidae fishes are a monophyletic family separated from the Characidae family (Buckup, 1998). In this, *Characidium* are the most diversified genus. Widely distributed throughout South American freshwater systems, this genus consists of small fish that rarely exceed a standard length (L_S) of 100 mm (Fig. 1). Despite a constant diploid number of chromosomes ($2n = 50$), *Characidium* fishes often have a karyotype variation concerning the chromosome formula, with the presence of B-chromosomes and location and number of the rDNA sites (Miyazaua & Galetti Jr., 1994; Maistro *et al*., 1998; Centofante *et al*., 2001; Vicari *et al*., 2008). Moreover, a highly differentiated ZZ/ZW sex chromosome system has been described in several *Characidium* species and populations (Vicari *et al*., 2008). In contrast to higher vertebrates, heteromorphic sex chromosomes are not a plesiomorphic condition in fish, having an independent origin within some genera and families, and this ZZ/ZW sex chromosome system in the *Characidium* genus appears to be a synapomorphy (Galetti Jr. & Foresti 1986; Artoni *et al*., 2001; Artoni & Bertollo, 2002; Centofante *et al*., 2002; Vicari *et al*., 2008). Chromosome data of the species *Characidium lanei* Travassos (Travassos, 1967) from the Atlantic basin in south Brazil are presented in this work, including an undescribed form of ZZ/ZW sex chromosome system. The presence of rDNA sites on sex chromosomes and their possible origin within the genus are briefly discussed.

†Author to whom correspondence should be addressed. Tel.: +55 41 3361 1749; fax: +55 41 3361 1793; email: rafanoleto@yahoo.com.br

FIG. 1. Specimen of *Characidium lanei* from Barroca river, Atlantic basin, PR, Brazil. Bar = 10 mm.

A total of 13 individuals of *C. lanei* (five males and eight females) from the Barroca river, Atlantic basin (25°24′40″ S; 48°50′28″ W), Paraná State, Brazil, were analysed. Chromosomal preparations were obtained from anterior kidney cells using the *in vivo* colchicine treatment (Bertollo *et al*., 1978). Constitutive heterochromatin was analysed by the C-banding method (Sumner, 1972) as well as by the double fluorochrome staining using chromomycin A_3+DAPI (Schweizer, 1980), which are indicative of GC and AT-rich regions, respectively. Nucleolar organizer regions (NOR) were identified by Ag-NOR staining (Howell & Black, 1980) and by fluorescent *in situ* hybridization (FISH) according to Heslop-Harrison *et al*. (1991). Double-FISH technique was performed with an 18S rDNA probe from the fish *Prochilodus argenteus* Spix & Agassiz (Hatanaka & Galetti Jr., 2004) and a 5S rDNA probe from the fish *Leporinus elongatus* Valenciennes (Martins & Galetti Jr., 1999). The probes 18S and 5S were labelled with biotin-14-dATP (Invitrogen; www.invitrogen.com) and digoxigenin-11-dUTP (Roche, www.roche.com), respectively, and both were obtained by polymerase chain reaction (PCR). The detection and amplification of hybridization signals were carried out using avidin–FITC conjugated (Sigma–Aldrich; www.sigmaaldrich.com) and anti-digoxigenin rhodamine (Roche). FISH signals were analysed in a Zeiss Axiophot epifluorescence microscope, and the chromosome images were captured by Case Data Manager Expo 4·0 (Applied Spectral Imaging; www.spectral-imaging.com) software.

Examined specimens of *C. lanei* presented the stable chromosome number in this genus with $2n = 50$ chromosomes. The number of chromosome arms (NF) equalled 98 in both sexes besides a female heterogamety related to a ZZ/ZW sex chromosome system (Fig. 2). In addition, this species revealed acrocentric chromosomes (pair 25). This fact indicates that rearrangements such as pericentric inversions are also involved in chromosomal diversification of this genus, although the presence of uniarmed elements is uncommon in *Characidium* species. The Z chromosome was a medium-sized metacentric, whereas W was a submetacentric element slightly larger than the Z. This sex system seems to be a synapomorphy restricted to species and populations from distinct hydrographic basins (Centofante *et al*., 2003; Maistro *et al*., 2004; Vicari *et al*., 2008).

C-banding showed the presence of heterochromatin in the centromeric regions of all chromosome pairs while the W chromosome appeared totally heterochromatic. In the Z chromosome, clear C-bands on both telomeric regions were detected [Figs 2(b,d) and 4(a)]. Heteromorphic terminal blocks were also observed on the

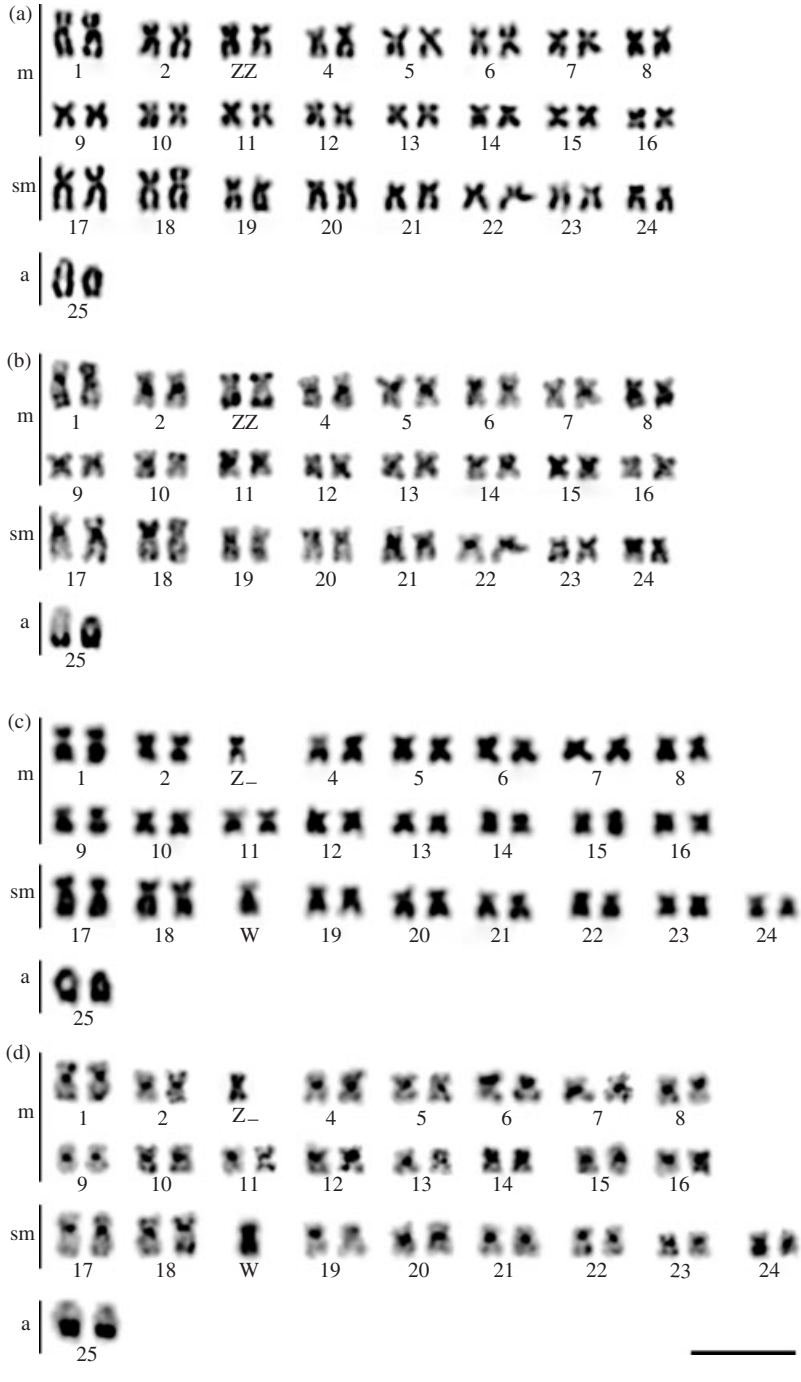

FIG. 2. Karyotypes of *Characidium lanei* arranged from giemsa-stained and sequential C-banding. (a, b) Male; (c, d) female. Bar = 5 μm.

long arms of the acrocentric pair. The telomeric regions are propitious to genetic material dispersion because of their proximity within interphase nucleus (Schweizer & Loidl, 1987) permitting heterochromatin transfers among sites of non-homologous chromosomes. In this sense, 4′, 6 diamidino-2-phenylindole dihydrochloride (DAPI) staining revealed heterochromatic domains with the same base composition (AT-rich) as the blocks on acrocentric and Z chromosomes [Fig. 3(a)]. In the latter case, the double chromomycin A3 (CMA_3)–DAPI staining also revealed a compartmentalization in AT and GC base pairs [Fig. 4(c)].

The major rDNA sites were visualized by Ag-NORs and 18S rDNA probe on the telomeric region of the short and long arms of the Z and W chromosomes, respectively [Fig. 4(b,d)], besides them to be GC rich [Fig. 4(c)]. The 5S ribosomal genes were located in the pericentromeric region of the short arm of an unidentified submetacentric pair [Fig. 3(b)]. The same condition was observed in other *Characidium* species (Vicari *et al*., 2008).

Concerning sex chromosomes, the heterochromatin distribution in Z chromosomes differs from the usual pattern in *Characidium* species, which is restricted to the pericentromeric region (Vicari *et al*., 2008). In addition, this study provides the first description of the rDNA sites on sex chromosomes in the genus. NORs located on sex chromosomes are only found in a few fish species (Morán *et al*., 1996; Reed & Phillips, 1997; Born & Bertollo, 2000). It was reported that in genus *Triportheus*, the rDNA possibly influenced the initial steps of the sex chromosome differentiation (Artoni & Bertollo, 2002). In *C. lanei*, however, it seems that other classes of repetitive DNA are involved in the evolution of such chromosomes because the location of rRNA genes on sex chromosomes represents a derived condition for the genus. Given that NORs in species of *Characidium* with sex chromosomes occur exclusively on the long arm of an autosomal submetacentric pair (Centofante *et al*., 2003), two hypotheses could be considered: (1) a translocation or transposition event

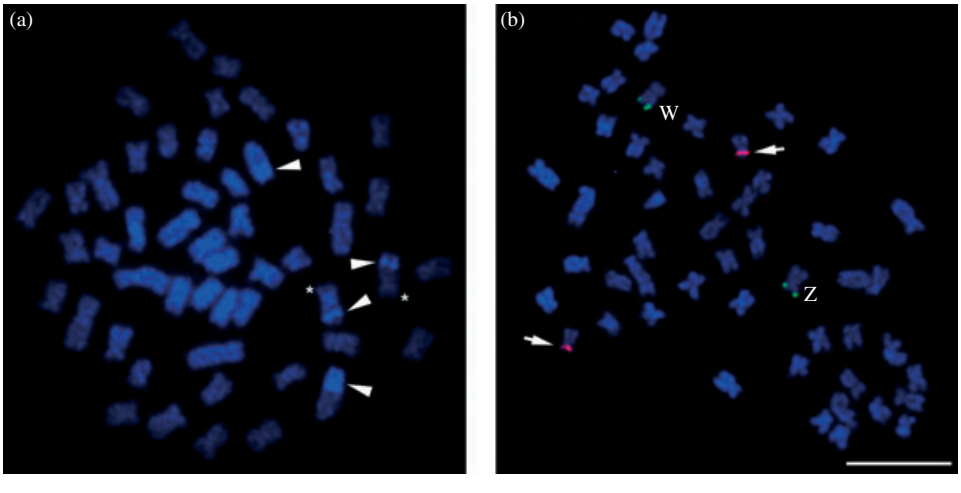

FIG. 3. (a) Metaphase of *Characidium lanei* male after DAPI staining. The arrowheads indicate AT-rich sites including on Z chromosomes (*). (b) Double fluorescent *in situ* hybridization (FISH) technique in female with 5S (arrows) and the 18S (on sex chromosomes) rDNA probes. Bar = 5μ m.

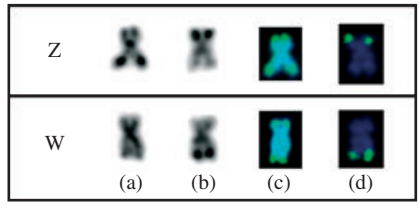

FIG. 4. The ZW sex chromosome pair of the *Characidium lanei*: (a) C-banding, (b) Ag-NOR sites, (c) CMA_3-DAPI staining and (d) 18S rDNA fluorescent *in situ* hybridization (FISH).

followed by sex chromosome diversification or (2) an old condition already present in the ancestral sex chromosome pair that was possibly lost in some species.

Given the current allopatric status of the ZW sex system in *Characidium* species, further analysis involving isolation and characterization of the heterochromatic segments as well as probes of sex chromosomes will provide a better understanding of sex chromosome diversification of this fish group.

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