Genetica (2008) 133:109–112
DOI 10.1007/s10709-007-9194-x

# Occurrence of ZZ/ZW sex chromosomes in *Thoracocharax stellatus* fish (Characiformes, Gasteropelecidae) from the Araguaia River, South America

**Paulo Cesar Venere · Issakar Lima Souza · Cesar Martins · Claudio Oliveira**

Received: 17 May 2007 / Accepted: 17 August 2007 / Published online: 3 September 2007


**Abstract** The karyotypic and chromosomal characteristics of the hatchetfish *Thoracocharax stellatus* from the Araguaia River, Brazil (Araguaia-Tocantins basin) were analyzed using Giemsa, $AgNO_3$, and $CMA_3$ fluorescent staining, and C-banding. The diploid chromosome number was 54 and the karyotypes of females and males were composed of six metacentrics, six submetacentrics, six subtelocentrics and 36 acrocentrics. Two unpaired acrocentric chromosomes were detected in the female karyotype. C-banding showed heterochromatic blocks at several chromosomes and an entirely heterochromatic acrocentric chromosome in females that was lacking in the male karyotype. This discovery indicated a heteromorphic sex chromosome system of the ZZ/ZW type. Ag-staining and $CMA_3$ fluorescence revealed one major chromosome pair bearing the NORs with the presence of additional signals in some metaphases. Both heterochromatic segments associated with Ag-NORs and the W chromosome were positively stained by $CMA_3$. Considering the present data and previous findings it is hypothesized that the occurrence of ZW sex chromosome system is widespread in the genus *Thoracocharax*.

**Keywords** Fish cytogenetics · Chromosome banding · Heteromorphic sex chromosomes · Hatchetfish

P. C. Venere (✉) · I. L. Souza
Instituto Universitário do Araguaia, Universidade Federal de Mato Grosso, Pontal do Araguaia, MT 78698-000, Brazil
e-mail: pvenere@uol.com.br

C. Martins · C. Oliveira
Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP 18618-000, Brazil

## Introduction

Gasteropelecidae (hatchetfishes) is a small freshwater fish family composed by three genera and nine species (Weitzman and Palmer 2003). Members of this family are commercially collected for the aquarium trade and about one million of units are sold every year in Brazil. Cytogenetic data available for gasteropelecids are restricted to the description of haploid number of *Carnegiella strigata* with $n = 25$–$26$ chromosomes (Hinegardner and Rosen 1972) or with $n = 24$ chromosomes (Scheel 1973) and *Gasteropelecus sternicla* with $n = 27$ chromosomes (Scheel 1973), and the karyotype of *Thoracocharax* cf. *stellatus* from the Amazon basin that presented $2n = 52$ chromosomes with the presence of a ZW sex chromosome system (Carvalho et al. 2002).

Although extensive karyotype diversity has been reported in the Neotropical fish fauna the occurrence of sex chromosomes is relatively rare and observed in about 5% of the species karyotyped (Oliveira et al. 2007), thus the occurrence of a ZW sex chromosome system in *Thoracocharax* cf. *stellatus* (Carvalho et al. 2002) represents an interesting starting point to investigate the distribution of this type of sex chromosomes in other gasteropelecids. In the present paper we investigated the karyotype of *Thoracocharax stellatus* (Kner, 1858) from the Araguaia River (Araguaia-Tocantins basin) with the aim to increase the cytogenetic data for the family and to evaluate the importance of chromosome rearrangements, including sex chromosome differentiation, in the evolution of gasteropelecids.

## Materials and methods

Mitotic chromosomes of *T. stellatus* (nine females and eight males) collected in the Araguaia River, Mato Grosso, Brazil were studied. The fishes were identified and voucher specimens were deposited in the fish collection of the Ichthyology Laboratory of the Departamento de Ciências Biológicas e da Saúde, Instituto Universitário do Araguaia, UFMT, Mato Grosso, Brazil (ICLMA 015). Chromosome preparations were obtained from kidney cells, by direct (Bertollo et al. 1978) and short-term culture methods (Fenocchio et al. 1991), and analyzed after conventional and differential staining. The chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST) or acrocentric (A), as frequently used on fish karyotype descriptions following the ratios proposed by Levan et al. (1964). NORs (Nucleolus Organizer Regions) were identified by silver staining (Ag-NORs), as described by Howell and Black (1980). C-banding and Chomomycin $A_3$ ($CMA_3$) staining were performed according to Sumner (1972) and Schmid (1980), respectively. Sequential $CMA_3$, C-banding and Ag-NOR staining was also performed.

## Results

The diploid number of *T. stellatus* was $2n = 54$ chromosomes for both sexes. The karyotypes of females and males were composed of 6 M, 6 SM, 6 ST and 36 A chromosomes (Fig. 1). The Ag-NORs were observed on the long arm of pair 15 with the presence of additional sites on the long arm of one medium sized acrocentric chromosome pair (Figs. 2a, b). The constitutive heterochromatin was distributed over several chromosomes, in pericentromeric and mainly in terminal positions (Fig. 2c). The long arms of chromosome pair 15 (Ag-NORs-bearing) revealed a conspicuous heterochromatic block (Fig. 1). Additionally, only one medium-sized acrocentric chromosome, almost entirely heterochromatic, was observed in the female karyotype, characterizing a W chromosome in this species. Male karyotypes present two acrocentric chromosomes with a large heterochromatic pericentromeric segment, while the remaining portion of the short arm is euchromatic (Fig. 1). The correspondence among Ag-NOR, C-banding and $CMA_3$ signals were determined after sequential treatment of metaphases.

## Discussion

The only common characteristic identified in all cytogenetic studies on Gasteropelecidae is the occurrence of a large number of subtelocentric and acrocentric chromosomes (Hinegardner and Rosen 1972; Scheel 1973; Carvalho et al. 2002; present paper). This result is quite surprising since extensive studies in Neotropical characiform families have shown that these usually have more metacentric and submetacentric chromosomes (Oliveira et al. 2007).

The presence of $2n = 54$ in *T. stellatus* from the Araguaia-Tocantins basin is also interesting, since the sample identified as *T.* cf. *stellatus* from the Amazon basin exhibited $2n = 52$ chromosomes (Carvalho et al. 2002). The occurrence of this conspicuous chromosome differentiation between both samples suggests that they are isolated and possibly represent different species.

The occurrence of Ag-NORs on the long arms of a chromosome pair (sometimes up to four chromosomes) of *T. stellatus* (present paper) reinforces the hypothesis that this sample is different from the sample of *T.* cf. *stellatus* from the Amazon River basin that presented Ag-NORs in a terminal position of the short arms of a subtelocentric chromosome pair (Carvalho et al. 2002). The analysis with $CMA_3$ showed that the heterochromatic segments associated with Ag-NORs and the W chromosome were positively stained suggesting the presence of a high number of GC bases in these chromosomes.

Besides the remarkable karyotypic differences identified between the samples of *T. stellatus* (present paper) and *T.* cf. *stellatus* (Carvalho et al. 2002), one additional difference was identified in relation to the sex chromosomes. Although both species share a ZZ/ZW system, in *T.* cf. *stellatus* from the Amazon basin the W chromosome is a very small acrocentric almost entirely heterochromatic, while in the *T. stellatus* from the Araguaia-Tocantins River basin the W is a medium-sized acrocentric fully heterochromatic.

The evolutionary process of the sex chromosomes seems to follow independent models in different fish groups. Thus, in some species of Poeciliidae and Hypoptopomatinae, the cytological differentiation of the sex chromosomes appears to be limited to the accumulation of heterochromatic segments. In other cases, such as in the classical ZZ/ZW described for various *Leporinus* species, an initial heterochromatinization seems to have been followed by a process of accumulation of heterochromatic segments, with the consequent increase in the size of the W sex chromosome in relation to the original homologues (for review, Venere et al. 2004). The occurrence of almost entirely heterochromatic W chromosomes in *T.* cf. *stellatus* (Carvalho et al. 2002) and *T. stellatus* (present paper) suggests that in the genus *Thoracocharax* the development of the W chromosome occurred by the accumulation of heterochromatin in these chromosomes.

Considering that *T. stellatus* is widely distributed throughout the Amazon and Orinoco basins (Weitzman and Palmer 2003) and strong barriers to their movements (like waterfalls) are not found in their distribution range, the cytogenetic differences found could be originated by a mechanism of distance isolation or, alternatively, be due

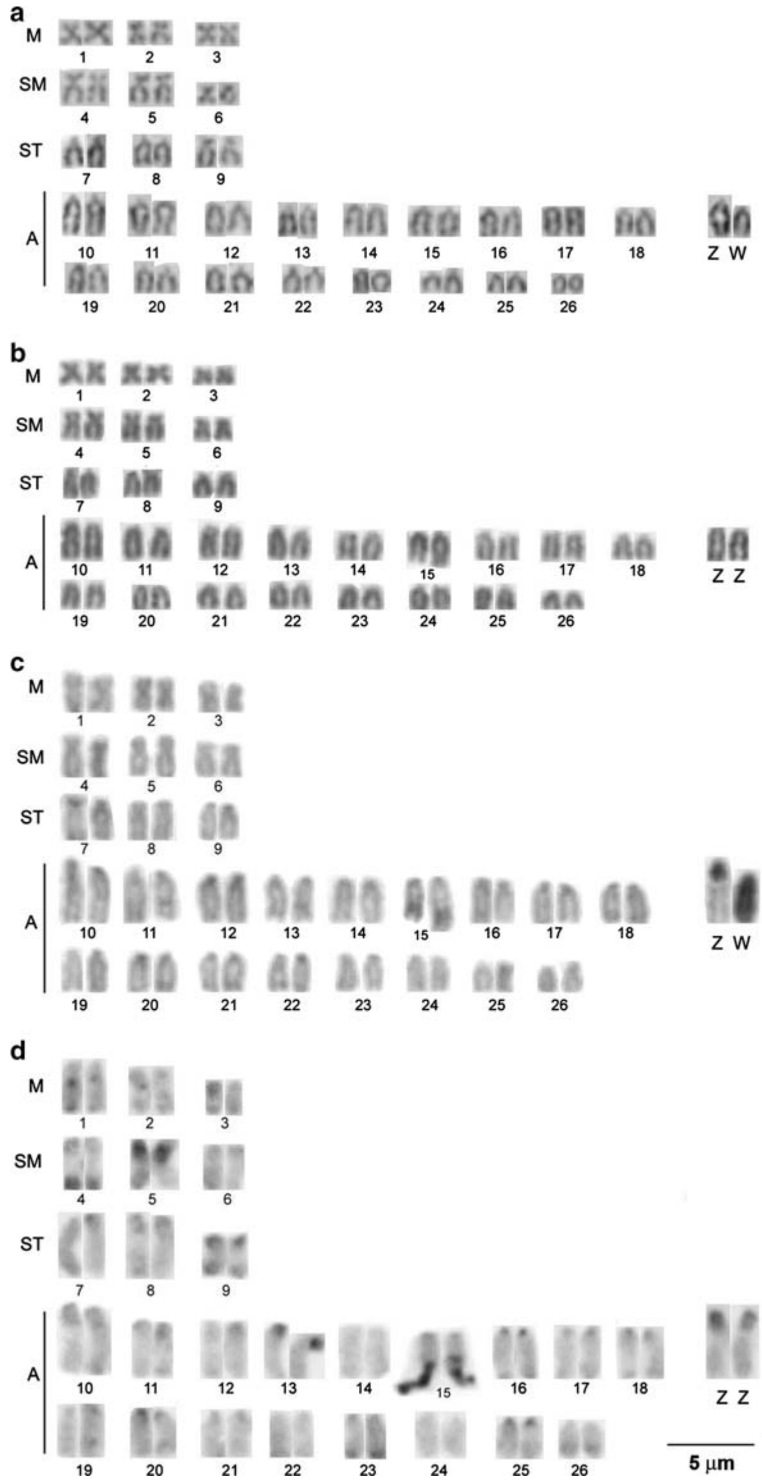


**Fig. 1** Female (**a**, **c**) and male (**b**, **d**) karyotype of *T. stellatus* arranged from conventionally Giemsa stained (**a**, **b**) and C-banded (**c**, **d**) chromosomes

historical events. The Araguaia-Tocantins river system where the present sample of *T. stellatus* were collected belongs to the Amazon Basin, however, this hydrographic system differ from other systems in Amazon because it flows directly in the estuary besides the Marajó Island experiencing a strong influence of Atlantic Ocean while the other river systems flow in the Solimões-Amazon Rivers. Although many species found in the Araguaia-Tocantins river system are also present in other Amazon rivers this hydrographic particularity is reflected in its fish fauna with many endemic species (Santos et al. 2004; Kullander and Ferreira 2006). Thus, the cytogenetic differences observed

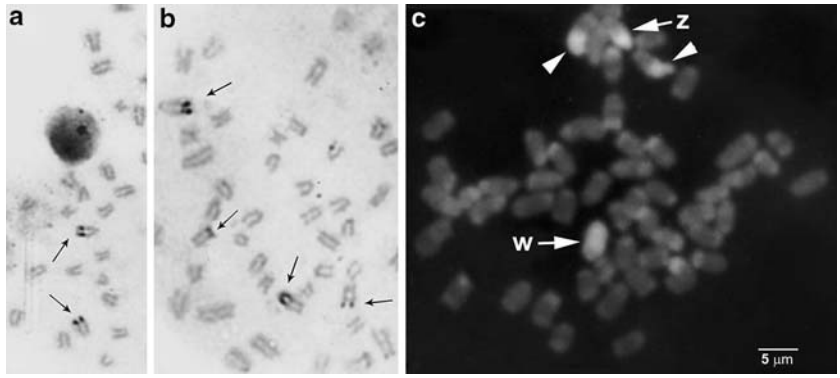


**Fig. 2** Ag-NORs (**a**, **b**) and $CMA_3$ fluorescence (**c**) staining of metaphasic chromosomes of *T. stellatus*. The arrows indicate a single Ag-NOR bearing chromosome pair (**a**), multiple Ag-NOR sites (**b**) and the Z and W chromosomes (**c**). The arrowhead indicates the GC-rich heterochromatin corresponding to Ag-NOR sites (**c**). (**a**) and (**b**) are partial metaphases

between the samples of *T. stellatus* (present paper) and *T.* cf. *stellatus* (Carvalho et al. 2002) may represent an additional example of species differentiation in the Araguaia River.

Eigenmann (1910) suggested that the fishes of the family Gasteropelecidae could constitute the sister group of the characid subfamilies Triportheinae and Cynodontidae. On the other hand, Weitzman (1960) suggested that the morphological similarity among Gasteropelecidae, Triportheinae and Cynodontidae might represent only an adaptive convergence. The presence of a ZZ/ZW sex chromosome system in *Thoracocharax* and in all species of *Triportheus* karyotyped (Artoni and Bertollo 2002; Nirchio et al. 2007), mainly the occurrence of an entirely heterochromatic W chromosome in *Triportheus venezuelensis* (Nirchio et al. 2007), reinforces the hypothesis that the family Gasteropelecidae may be related to the genus *Triportheus*. Additionally, heterochromatinization of the W chromosome in *Thoracocharax* seems to be associated with a reduction in its size during the evolution of the ZW sex system, as suggested for *Triportheus* (Artoni and Bertollo 2002; Nirchio et al. 2007). Additional cytogenetic studies in different samples of *Thoracocharax* and other genera of Gasteropelecidae will be very useful for a better known about species and chromosome differentiation in this family.

**Acknowledgements** The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMAT (Fundação de Amparo a Pesquisa de Mato Grosso) for their financial support and Jason L. Oliveira and Humberto P. Rego for their assistance during fish capture.

## References

Artoni RF, Bertollo LAC (2002) Evolutionary aspects of the ZZ/ZW sex chromosome system in the Characidae fish, genus *Triportheus*. A Monophyletic state and NOR location on the W chromosome. Heredity 89:15–19

Bertollo LAC, Takahashi CS, Moreira-Filho O (1978) Cytotaxonomic consideration on *Hoplias lacerdae* (Pisces, Erythrinidae). Braz J Genet 1:103–120

Carvalho ML, Oliveira C, Foresti F (2002) Description of a ZZ/ZW sex chromosome system in *Thoracocharax* cf. *stellatus* (Teleostei, Characiformes, Gasteropelecidae). Genet Mol Biol 25:299–303

Eigenmann CH (1910) Catalogue of the freshwater fishes of tropical and south temperate America. III Zoology. Reports of the Princeton University Expeditions to Patagonia, 1896–1899. Princeton, pp 375–511

Fenocchio AS, Venere PC, Cesar ACG, Dias AL, Bertollo LAC (1991) Short term culture from solid tissues of fishes. Caryologia 44:161–166

Hinegardner R, Rosen DE (1972) Cellular DNA content and evolution of teleostean fishes. Amer Nat 106:621–644

Howell WM, Black DA (1980) Controlled silver staining nucleolus organizer regions with protective colloidal developer: a 1–step method. Experientia 36:1014–1015

Kullander SO, Ferreira EJG (2006) A review of the South American cichlid genus *Cichla*, with descriptions of nine new species (Teleostei: Cichlidae). Ichthyol Explor Freshwaters 17:289–398

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220

Nirchio M, Oliveira C, Ferreira IA, Granado A, Ron E (2007). Extensive polymorphism and chromosomal characteristics of ribosomal DNA in the characid fish *Triportheus venezuelensis* (Characiformes, Characidae). Genet Mol Biol 30:25–30

Oliveira C, Almeida-Toledo LF, Foresti F (2007) Karyotypic evolution in Neotropical fishes. In: Pisano E, Ozouf-Costaz C, Foresti F, Kappor BG (eds) Fish cytogenetics. Science Publisher, Enfield, NH, USA

Santos GM, Mérona B, Juras AA, Jégu M (2004) Peixes do baixo rio Tocantins: 20 anos depois da Usina Hidrelétrica Tucuruí. Eletronorte, Brasília, Brazil

Scheel JJ (1973) Fish chromosome and their evolution. Internal Report of Denmarks Akvarium, Charlottenlund, Denmark 22

Schmid M (1980) Chromosome banding in Amphibia. IV. Diferentiation of GC- and AT- rich chromosome regions in Anura. Chromosoma 77:83–103

Sumner AT (1972) A single technic for demonstrating centromere heterochromatin. Exptl Cell Res 75:304–306

Venere PC, Ferreira IA, Martins C, Galetti PM Jr (2004) A novel ZZ/ZW sex chromosome system for the genus *Leporinus* (Pisces, Anostomidae, Characiformes). Genetica 121:75–80

Weitzman SH (1960) The phylogenetic relationships of *Triportheus*, a genus of South American characid fishes. Stanf Ichth Bull 7:239–243

Weitzman SH, Palmer L (2003) Family Gasteropelecidae (freshwater hatchetfishes). In: Reis RE, Kullander SO, Ferraris SJ (eds) Check list of the freshwater fishes of South and Central America. EDIPUCRS, Porto Alegre, Brazil