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A ZZ/ZW sex chromosome system in Cheirodontinae fish

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Abstract. Cytogenetic studies were carried out on two Neotropical fish species of the subfamily Cheirodontinae. *Odontostilbe paranensis* and *Holoshestes heterodon*, from Mogi-guaçu River (Brazil), were characterized by 2n = 52 chromosomes. Males of *O. paranensis* showed 18 meta-submetacentric, six subtelocentric and two acrocentric chromosome pairs, resulting in a fundamental number of 100. Females of this species presented a karyotype composed of 18 meta-submetacentric pairs, six subtelocentric pairs, one acrocentric pair and also one medium submetacentric chromosome and one medium acrocentric element. The karyotype of females of *H. heterodon* presented 18 meta-submetacentric, six subtelocentric and one acrocentric chromosome pairs, and two single small chromosomes - a metacentric one and an acrocentric element. A fundamental number of 101 was detected on females of both species. The present results suggest the occurrence of ZZ/ZW sex chromosomes on these two species, characterized by an acrocentric Z chromosome and by a morphologically variable W chromosome.

Keywords : sex chromosomes, ZZ/ZW, Fish, Cheirodontinae

Introduction

The subfamily Cheirodontinae comprises a fish group of approximately 40 genera and 90 species of small size that show wide geographical distribution throughout Central and South America (Gery, 1977). Despite the great number of species and their broad distribution, the taxonomy of these characids is so far poorly defined (Malabarba, 1988) and there are still few chromosome data on this group (for revision Oliveira *et al.*, 1988). The diploid chromosome number is only known for *Cheirodon axelrodi*, *Paracheirodon innesi* (Gyldenholm and Scheel, 1971), *Probolodus heterostomus* (Paganelli *et al.*, 1987), *Cheirodon notomelas*, *Cheirodon* sp. (Nishiyama and Santos, 1995) and *Odontostilbe claudiae* (Cesar and Galetti, 1990).

Recent cytological advances have improved the information on fish chromosomes. An extensive karyotype diversity has been reported in the Neotropical fish fauna and, even though fish usually do not present morphological differentiated sex chromosomes, several studies have been demonstrating their occurrence in different groups, specially in freshwater species. Different male or female heterogamety can be found on Neotropical fish species (*e.g.* Moreira Filho *et al.*, 1993) and the ZZ/ZW system has been described on some groups, as Anostomidae (Galetti *et al.*, 1981), Characidae (Bertollo and Cavallaro, 1992 ; Maistro *et al.*, 1998), Prochilodontidae (Feldberg *et al.*, 1987) and Parodontidae (Moreira Filho *et al.*, 1993).

In the present study, the karyotypes of two freshwater Cheirodontinae fish species (Characiformes, Characidae), *Odontostilbe paranensis* and *Holoshestes heterodon*, are described and the occurrence of differentiated sex chromosomes is suggested for both species.

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Materials and methods

Individuals of *Odostostilbe paranensis* (seven females and two males) and two female specimens of *Holoshestes heterodon* were collected in an oxbow pond of Mogi-guaçu River (municipality of Luiz Antônio, São Paulo State, Brazil). Mitotic chromosomes were obtained from kidney suspension cells and Giemsa-stained as described in Bertollo *et al.* (1978). The chromosomes were classified as metacentric (M), submetacentric (SM), subtelocentric (ST) and acrocentric (A) (Levan *et al.*, 1964).

Results

All analyzed specimens of *Odontostilbe paranensis* and *Holoshestes heterodon* presented a karyotype with a diploid number equal to 52 chromosomes (Fig. 1). In *O. paranensis*, the male individuals showed 26 homologous pairs comprising 18 meta-submetacentric, six subtelocentric and two acrocentric chromosome pairs, with a fundamental number (FN) of 100 (Fig. 1a), while all studied females presented 25 homologous pairs (18 meta-submetacentric, six subtelocentric and one acrocentric pair) plus two heterologous chromosomes, a medium-sized submetacentric and an acrocentric one, with FN = 101 (Fig. 1b). These karyotype differences between males and females of *O. paranensis* suggest the occurrence of a ZZ/ZW sex chromosome mechanism in this species. The single acrocentric chromosome observed on females should correspond to the pair 26 of the males (chromosomes Z) and the single submetacentric chromosome that seems to be exclusive to the female karyotype should represents a W element. Of note, the Z chromosome showed an evident terminal secondary constriction on the short arm (Fig. 1a,b).

The 2 females of *Holoshestes heterodon* presented 18 meta-submetacentric pairs, six subtelocentric pairs, one acrocentric pair and two small single chromosomes, a metacentric one and an acrocentric element (Figure 1c), with a fundamental number of 101.

Discussion

The karyotypes of the two analysed species are characterised by the same diploid chromosome number (2n = 52). Although Cheirodontinae species present distinct numbers of chromosomes (*e.g.* Oliveira *et al.*, 1988), most studied species of the group also showed a karyotype with 52 chromosomes, as *Cheirodon axelrodi* (Gyldenholm and Scheel, 1971), *Cheirodon notomelas*, *Cheirodon* sp. (Nishiyama and Santos, 1995) and *Odontostilbe claudiae* (Cesar and Galetti, 1990). The presence of the same diploid number on at least three different genera can indicate a partial stability of the chromosome structure in Cheirodontinae, although differences in the chromosome morphology among karyotypes suggest the occurrence of several rearrangements among species. Differences in the number of chromosomes among other species of this subfamily could also be due to chromosome rearrangements or, since Cheirodontinae can represent a polyphyletic group of species (*e.g.* Malabarba, 1988), these differences could even be related to taxonomic problems.

Male individuals of *Odontostilbe paranensis* presented 18 meta-submetacentric, six subtelocentric and two acrocentric chromosome pairs, while females showed 18 meta-submetacentric, 6 subtelocentric and one acrocentric chromosome pairs plus a single medium submetacentric chromosome and a single medium acrocentric element. The observed karyotype differences on males and females of *O. paranensis* suggest the presence of a ZZ/ZW sex chromosome system - males are homogametic (ZZ) and females are heterogametic (ZW). The putative chromosomes W and Z showed to be highly differentiated in morphology and size.

Although only females of *Holoshestes heterodon* were available for analysis, data on this species also indicate a chromosome polymorphism that could be related to a population variability or even to a sex differentiation. It seems probable that this species could also present a ZZ/ZW sex chromosome mechanism, where the Z chromosome corresponds to a small acrocentric and the W chromosome corresponds to a small metacentric.

The presence of nucleolar organizer regions (NORs) on secondary constrictions is a common feature on several eukariotes (Warburton and Henderson, 1979). The terminal secondary constrictions on the short arm of a medium acrocentric chromosome in *O. paranensis* (chromosome Z) could be related to NOR sites. Although the presence of NORs on sex chromosomes has already been described on some fish species (*e.g.* Born and Bertollo, 2000), it seems to be a character that is not so common. The significance and the variability of the NORs in *O. paranensis* have been currently investigated by silver nitrate staining and FISH (Fluorescence *in situ* hybridization).

Although sex chromosomes have been described in several Neotropical fish groups (*e.g.* Moreira Filho *et al.*, 1993; Born and Bertollo, 2000), their occurrence among Characidae species is described only on Triportheinae (Bertollo and Cavallaro, 1992) and Characidiinae (Maistro *et al.*, 1998). The possible ZZ/ZW sex chromosome system in *Odontostilbe paranensis* and *Holoshestes heterodon* may indicate that the appearance of cytologically differentiated sex chromosomes could have occurred several and independent times during the evolution of characids. It would be particularly interesting to improve cytogenetic data on Cheirodontinae and compare the present results with those of other related species.

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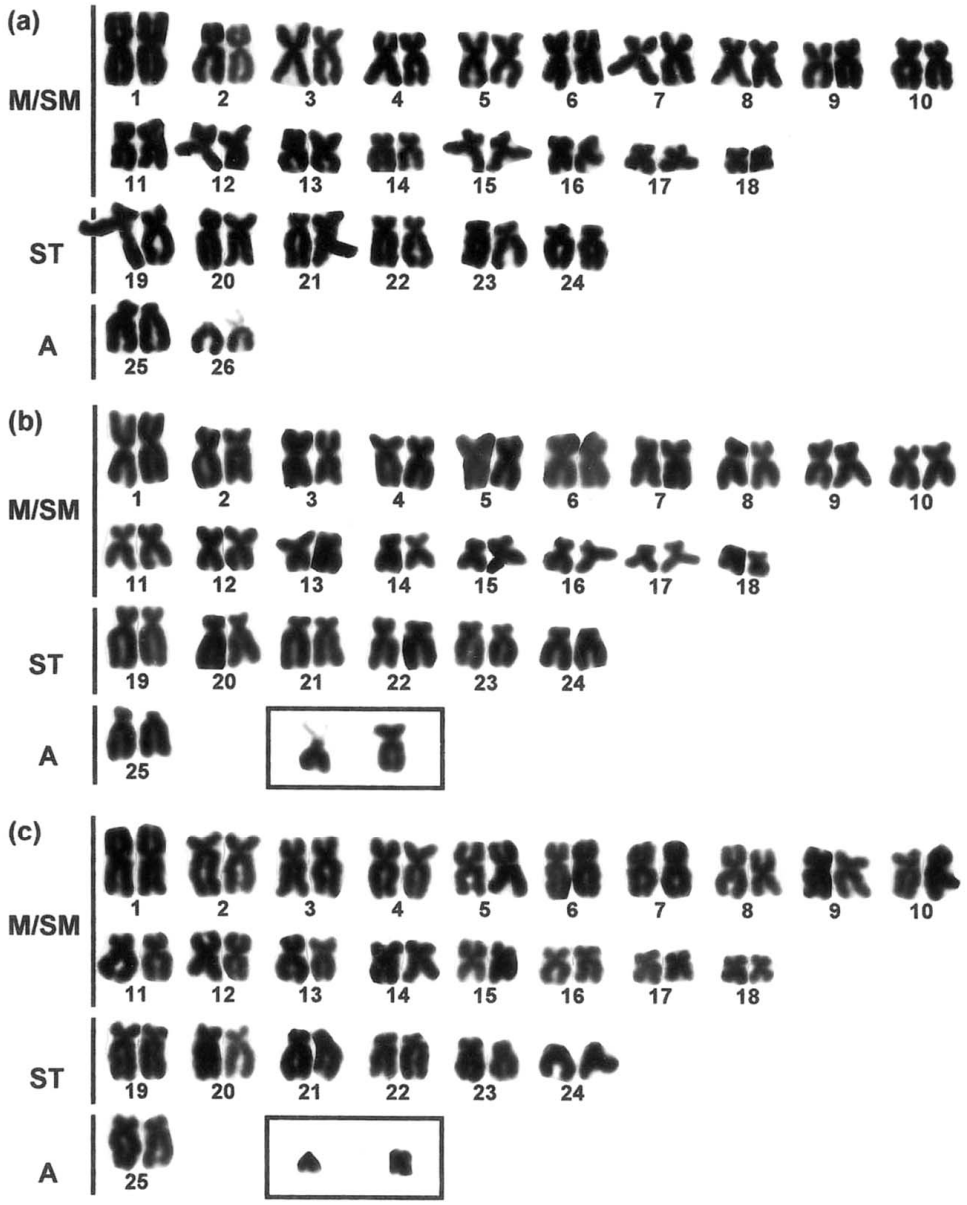

Figure 1. Karyotypes of the analyzed species. (a) male of *Odontostilbe paranensis* ; (b) female of *Odontostilbe paranensis*, and (c) female of *Holoshestes heterodon.*

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