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Cytogenetic analysis and description of the sexual chromosome determination system ZZ/ZW of species of the fish genus *Serrapinnus* (Characidae, Cheirodontinae)

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ABSTRACT. Four populations of *Serrapinnus notomelas* and one population of *Serrapinnus* sp.1, both belonging to the subfamily Cheirodontinae, were analyzed by Giemsa and silver nitrate impregnation techniques. We found 2n = 52 chromosomes for all populations, with interspecific differences in the karyotype formula; *S. notomelas* showed 16m + 22sm + 10st + 4a, with fundamental number (FN) = 100 for males, and 16m + 23sm + 10st + 3a, with FN = 101 for females. *Serrapinnus* sp.1 had 8m + 16sm + 4st + 24a, with FN = 80 for males, and 8m + 15sm + 4st + 25a, with FN = 79 for females. The difference in FN for the two sexes is due to a pair of heteromorphic chromosomes in the females of both species, which characterizes a ZZ/ZW-type mechanism of chromosome sexual determination. Interspecies differences were also found in nucleolus organizer regions (NORs). A simple NOR system was detected in three of four *S. notomelas* populations, while *Serrapinnus* sp.1 had two chromosome pairs with NOR. Although *S. notomelas* and *Serrapinnus* sp.1 have the same diploid number, differences in the karyotype structure indicate that these are different species. Apparently there was pericentric inversion during the karyotype evolution of these species.

Key words: Karyotype, Nucleolus organizer region band,
Sexual chromosome ZZ/ZW, *Serrapinnus notomelas*, *Serrapinnus* sp

INTRODUCTION

Fish of the Cheirodontinae subfamily are less than 10 cm long and are common in marginal vegetation of all Brazilian lotic and lentic environments (Buckup and Malabarba, 1983). Systematic classification of this subfamily presents divergences and has been widely discussed. Gregory and Conrad (1938) stated that Tetragonopterinae may have derived from the Cheirodontinae subfamily or they may have had a common ancestor. While Weitzman and Fink (1983) discussed weaknesses in the classification of Neotropical characids, Weitzman and Vari (1988) did not accept Cheirodontinae as separate from Tetragonopterinae. Although this classification is still not universally accepted, Malabarba (1998) redefines Cheirodontinae as a subfamily comprising 20 genera, including *Serrapinnus*.

Although the ichthyofauna of continental waters in Neotropical regions may comprise up to 8,000 species (Vari and Malabarba, 1998), knowledge on their cytogenetics is still scarce.

Serrapinnus notomelas is the only species of the genus *Serrapinnus* reported to occur in the Paraná River basin. The distinctive characteristic of this species is darkly colored anterior portions of the dorsal and anal fins. However, two other morphotypes have been described: *Serrapinnus* sp.1, with dorsal and anal hyaline fins, and *Serrapinnus* sp.2, with dorsal and anal fins with only a dark patch on their extremities (Graça, 2004). These are probably different species.

We made cytogenetic analyses of *S. notomelas* and *Serrapinus* sp.1 collected from Tietê and Paraná River basins.

MATERIAL AND METHODS

Specimens from four populations of *S. notomelas* from the Paraná River, Cascatinha stream and Araquá River in Botucatu, SP region and from the Pântano River in São Carlos, SP region (Tietê River basin) were analyzed. Specimens of *Serrapinnus* sp.1 were collected in the Paraná River, in Porto Rico region. Mitotic chromosomes were obtained from kidney cells, following methodology described by Bertollo et al. (1978). Nucleolus organizer regions (NORs) were identified by silver nitrate staining (Howell and Black, 1980). Chromosomes were identified according to arm ratio criteria, suggested by Levan et al. (1964), as follows: metacentric chromosomes (m); submetacentric chromosomes (sm); subtelocentric chromosomes (st), and acrocentric chromosomes (a).

RESULTS

Serrapinnus notomelas showed a diploid number of 52 chromosomes for all populations. The karyotype was 16m + 22sm + 10st + 4a, with fundamental number (FN) = 100 for males (Figure 1A) and 16m + 23sm + 10st + 3a, with FN = 101 for females (Figure 1B). A female heterogamete system (ZZ/ZW) is thus characterized. Size differences between the first and second pair of chromosomes were found. An NOR was detected on the short arm of an acrocentric chromosome pair (pair 26) in populations of Pântano and Araquá Rivers and from Cascatinha stream (Figure 1). Variations of one to eight chromosome pairs with rDNA genes from the Paraná River population were found (data not shown). *Serrapinnus* sp.1 showed a diploid number of 2n = 52 chromosomes, with karyotype formula 8m + 16sm + 4st + 24a, and FN = 80 for males (Figure 2A), and 8m + 15sm + 4st + 25a, with FN = 79 for females (Figure 2B). NORs were found in one of the homologues of pair 19 and in both chromosomes of pair 20 (Figure 2). These NORs are strongly marked and heteromorphic in size; a multiple NOR system was demonstrated.

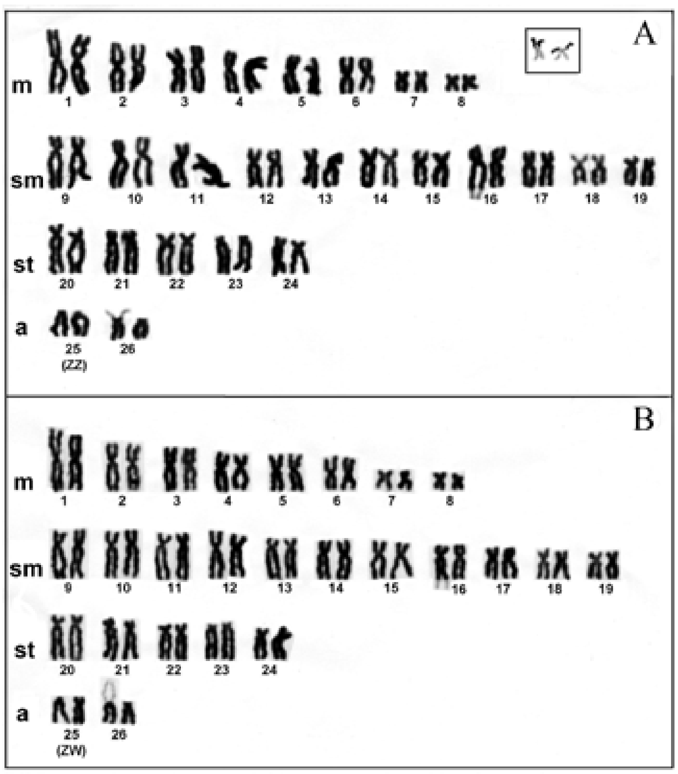

Figure 1. Karyotype of *Serrapinnus notomelas* of the Paraná River basin, from populations of Araquá and Pântano Rivers and Cascatinha stream. **A.** Male. **B.** Female. Nucleolus organizer region-bearing chromosomes (pair 26) in detail. m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric.

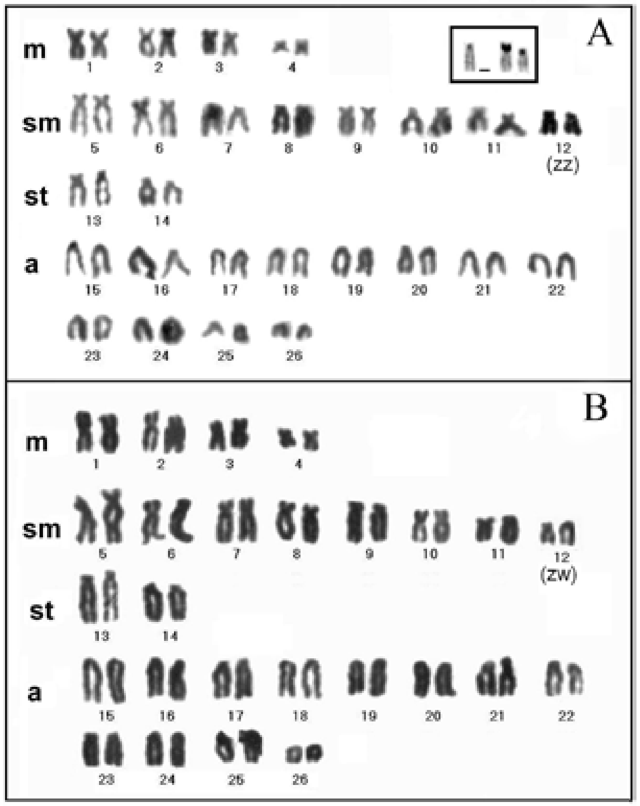

Figure 2. Karyotype of *Serrapinnus* sp.1. **A.** Male. **B.** Female. Nucleolus organizer region-bearing chromosomes (pair 26) in detail. m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric.

DISCUSSION

All *S. notomelas* populations had the same diploid number and the same karyotype constitution. Although it had the same diploid number, *Serrapinnus* sp.1 was different from *S. notomelas* in karyotype constitution. *S. notomelas* had a larger number of submetacentric (11 pairs) and a smaller number of acrocentric chromosomes (3 pairs), whereas *Serrapinnus* sp.1 had a greater number of acrocentric chromosomes (12 pairs). Furthermore, we found size differences between the first and second pairs. While the first pair was found to be larger than the second in *S. notomelas*, this difference in *Serrapinnus* sp.1 was not significant. These differences characterize distinct species and indicate that structural rearrangements of the pericentric inversion type occurred during the speciation process. Intergenus variations in the diploid number of the subfamily Cheirodontinae have been reported, with 2n = 32 chromosomes in *Paracheirodon innesi* (Gyldenholm and Scheel, 1971) and 2n = 52 chromosomes for most species, at the interspecies level in *Cheirodon tronneri* (n = 25; Scheel, 1973), *C. axelroidi* (n = 24; Post, 1965) (n = 26; Scheel, 1973), and intraspecies in *P. innesi*, with 2n = 32 and 2n = 36 chromosomes (Gyldenholm and Scheel, 1971).

The two species that we examined presented the same diploid number and type of chromosomal sex determination mechanism, except for sex chromosome pair and type, which were different. Submetacentric and acrocentric W chromosomes are respectively accountable for female heterogametes in *S. notomelas* and *Serrapinnus* sp.1. Differences in the sexual pair may be due to pericentric inversions that accompanied the karyotype evolution process of these two species. Chromosome mechanisms of sex determination were not observed in other *Serrapinus* species.

Wasko et al. (2001) analyzed *Odontostilbe paranensis* and *Holoshestes heterodon*, both of the subfamily Cheirodontinae, and reported a diploid number of 52 chromosomes. *O. paranensis* has the same type of chromosomal sexual heteromorphism that we observed in the genus *Serrapinnus*. Similar to *S. notomelas*, this species presented a greater number of metacentric/submetacentric chromosome pair. Analysis of *H. heterodon* females also showed probable chromosomal sexual heteromorphism owing to one acrocentric/metacentric chromosome pair. However, Wasko and Galetti Jr. (1994) failed to observe karyotype differences between the sexes in *O. claudinae*. Based on these earlier papers and our research, this type of ZZ/ZW sexual chromosome mechanism is unique to this subfamily in the Characidae. Almeida-Toledo and Foresti (2001) demonstrated that such a system is more frequent among Neotropical fish. Morphologically differentiated ZZ/ZW-type sexual chromosomes in the Characid family have been reported in *Triportheus guintheri* (Bertollo and Cavallaro, 1992; Artoni et al., 2001; Artoni and Bertollo, 2002), *T. albus*, *T. elongatus* and *T. flavus* (Falcão, 1988), and in *T.* cf *elongatus* and *T. paranense* (Artoni et al., 2001; Artoni and Bertollo, 2002). Consequently, Cheirodontinae and Triportheinae are the only subfamilies with cytological evidence of heterogamety of sexual chromosomes among Characids. According to Galetti Jr. et al. (1981), sexual chromosome heteromorphism is common in fish. Moreira-Filho (1983) reported that 15.8% of fish species studied in Brazil have a sexual chromosome mechanism, ranging from simple heterogamety XX/XY and ZZ/ZW to cases of multiple mechanisms involving more than one chromosome pair ($X_1X_1X_2X_2/X_1X_2Y$, XX/XY_1Y_2 and ZZ/ZW_1W_2).

Simple AgNORs were observed in three of four populations of *S. notomelas.* The Paraná River population was found to be unique in current intra- and interindividual variations in the NOR distribution pattern identified by silver nitrate and FISH, indicating a transposition mechanism (data not shown). A simple NOR in the subfamily Cheirodontinae was reported by Pacheco and Miyazawa (2002) in *Odontostilbe pequira* from the Cuiabá River. Wasko and Galetti Jr. (1994) also detected a secondary constriction in the short arm of an acrocentric chromosome in *O. paranensis*, which may indicate an NOR site in this species.

Different from most of the *S. notomelas* populations (Pântano and Araquá Rivers, Cascatinha stream), a multiple NOR system with three chromosomes was observed by Ag-NOR technique in *Serrapinnus* sp1. One to three nucleoli in each interphase nucleus corroborate this finding. Pacheco and Miyazawa (2002) detected the same NOR distribution for *S. kriege*, with markings on two non-homologous chromosomes, and for *S. calliurus*, with four marked chromosomes.

Although this is one of the first reports on *Serrapinnus* species, cytogenetic studies on Cheirodontinae contribute not only to studies on the ZZ/ZW sexual chromosome mechanism involving W chromosome types proper to each species, but are also useful for determination of cytotaxonomic characteristics that differentiate species with synonymy.

REFERENCES

Almeida Toledo LF and Foresti F (2001). Morphologically differentiated sex chromosomes in Neotropical freshwater fish. *Genetica* 111: 91-100.

Artoni RF and Bertollo LA (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.

Artoni RF, Falcão JN, Moreira-Filho O and Bertollo LA (2001). An uncommon condition for a sex chromosome system in Characidae fish. Distribution and differentiation of the ZZ/ZW system in *Triportheus*. *Chromosome Res.* 9: 449-456.

Bertollo LA and Cavallaro ZI (1992). A highly differentiated ZZ/ZW sex chromosome system in a Characidae fish, *Triportheus guentheri*. *Cytogenet. Cell Genet.* 60: 60-63.

Bertollo LA, Takahashi CS and Moreira-Filho O (1978). Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Rev. Bras. Genet.* 2: 103-120.

Buckup PA and Malabarba LR (1983). A list of the fishes of the taim ecological station, Rio Grande do Sul, Brasil. *Iheringia Ser. Zool.* 63: 103-113.

Falcão JN (1988). Caracterização cariotípica em peixes do gênero *Triportheus* (Teleostei, Characiformes, Characidae). Doctoral thesis, Departamento de Genética e Matemática Aplicada à Biologia, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto.

Galetti PM Jr, Foresti F, Bertollo LA and Moreira-Filho O (1981). Heteromorphic sex chromosomes in three species of the genus *Leporinus* (Pisces, Anostomidae). *Cytogenet. Cell Genet.* 29: 138-142.

Graça WJ (2004). Caracterização morfológica dos peixes da planície de inundação do alto rio Paraná, MS. Master's thesis, Universidade Estadual de Maringá, Maringá.

Gregory WK and Conrad GM (1938). The phylogeny of the *Characin* fishes. *Zoologica* 23: 319-360.

Gyldenholm AO and Scheel JJ (1971). Chromosome number of fishes. *J. Fish Biol.* 3: 479-486.

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

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

Malabarba LR (1998). Monophyly of the Cheirodontinae, characters and major clades (Ostariophysi: Characidae). In: Phylogeny and classification of Neotropical fishes (Malabarba LR, Reis R, Vari R, Lucena ZMS, et al., eds.). EDIPUCRS, Porto Alegre, 193-233.

Moreira-Filho O (1983). Estudos na família Parodontinae (Pisces, Cypriniformes) da bacia do Passa-Cinco/SP: aspectos citogenéticos e considerações correlatas, São Carlos, SP. UFSCar, 1983. Master's thesis, Departamento de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos.

Pacheco EB and Miyazawa CS (2002). Estudos citogenéticos em Cheirodontinae (Characiformes, Characidae) do pantanal de Mato Grosso. In: IX Simpósio de Citogenética e Genética de Peixes, Maringá, 38.

Post A (1965). Vergeichende untersuchungen der chromosomenzahlen bei Susswasser Teleosteein. *Z. Zool. Syst. Evol. Forsch.* 3: 47-93.

Scheel JJ (1973). Fish chromosomes and their evolution. Interval report of Denmarks Akvarium, Charlottinlund.

Vari RP and Malabarba LR (1998). Neotropical ichthyology: an overview. In: Phylogeny and classification of Neotropical fishes (Malabarba LR, Reis R, Vari RP, Lucena ZMS, et al., eds.). EDIPUCRS, Porto Alegre, 1.

Wasko AP and Galetti PM Jr (1994). Citogenética de pequenos peixes caracídeos do grupo Cheirodontinae. Cromossomos mitóticos de *Odontostilbe paranensis*, *O. claudinae* e *Holoshestes heterodon*. In: Congresso Brasileiro de Genética, Caxambu, 122.

Wasko AP, Cesar ACG, Martins C and Galetti PM Jr (2001). A ZZ/ZW sex chromosome system in Cheirodontinae fish. *Chromosome Sci.* 5: 145-148.

Weitzman SH and Fink WL (1983). Relationships of the neon tetras, a group of South American freshwater fishes (Teleostei, Characidae) with comments on the phylogeny of new word Characiforms. *Bull. Mus. Comp. Zool.* 6: 339-395.

Weitzman SH and Vari RP (1988). Miniaturization in South American freswater fishes; an over-view and discussion. *Proc. Biol. Soc. Wash.* 2: 444-465.