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First chromosome data on *Steindachneridion scripta* (Pisces, Siluriformes, Pimelodidae) from Brazilian rivers: Giemsa, CBG, G-, and RE banding

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ABSTRACT. A cytogenetic study was performed on the large pimelodid species *Steindachneridion scripta* (Siluriformes, Sorubiminae) from the Paraná River basin (Brazil). Chromosome preparations were obtained avoiding sacrifice of the specimens, by means of lymphocyte culture, and several staining and chromosome banding techniques were applied. The karyotype consisted of 56 chromosomes, 24 metacentrics, 20 submetacentrics, 4 subtelocentrics, and 8 acrocentrics (fundamental number = 104). The first pair of acrocentric chromosomes (pair 25) consistently had a decondensed secondary constriction; the C-banding pattern of some chromosomes allows them to be considered cytogenetic markers (i.e., pairs 1, 3, 4, 6, 7, 9, 13, 23, and 24). G-banding and restriction enzymes provided patterns that helped improve chromosome pairing. This is the first report on a Neotropical pimelodid species of economic

interest using several cytogenetic techniques and providing an integral karyotypic characterization.

Key words: *Steindachneridion*, C-, B- and RE banding, Neotropical region

INTRODUCTION

The genus *Steindachneridion* belongs to the order Siluriformes and has a restricted distribution in certain South American river basins. To date, there are five known species within the genus; a common characteristic is the endemism of almost all of them: *S. scripta* from the Paraná and Uruguai Rivers, *S. doceana*, from the Doce River, *S. parahybae* from the Paraíba River, *S. amblyurus* from the Jequitinhonha River, and *Steindachneridion* sp from the Iguaçu River (Burguess, 1989; Lundberg and Littmann, 2003). Due to common morphological and anatomic features, *Steindachneridion* was included within the Sorubiminae - *sensu* Ringuelet (1967) - (Pimelodidae) with other large siluriform fishes of economic importance (i.e., *Pseudoplatystoma*, *Brachyplatystoma*, *Zungaro*). Commonly these fishes were called "surubins, pintados, douradas, jaús" and they are some of the largest of the Neotropical fish fauna that represent an important economic source for populations along South American river basins.

Steindachneridion (surubim, monjolo) species suffer intense anthropogenic pressure, through modification to their habitat and due to overfishing; consequently, some of them are highly endangered and practically extinct in various parts of their original areas of distribution, with few biological (reproductive, ethological, physiological, etc.) studies being available. Due to their economic importance as a food source, some aquaculture stations are currently developing programs for captive propagation of *Steindachneridion* sp from the Iguaçu River and *S. scripta* from the Paraná River. Fish produced by these programs would be used in fish farms as well to restore natural stocks in degraded habitats. We used several cytogenetic techniques to obtain an integral characterization of *S. scripta* from the Paraná River basin. The data obtained could be useful for cytotaxonomic, evolutionary, reproductive, and conservation studies.

MATERIAL AND METHODS

Seven adult specimens of *S. scripta* (five males, one female and one of indeterminate sex) (Figure 1) from the Upper Paraná basin (Paranapanema and Tibagi Rivers, Paraná State, Brazil) were studied cytogenetically. Mitotic chromosome preparations were obtained from lymphocyte culture following the method of Fenocchio and Bertollo (1988), avoiding the sacrifice of specimens. C- and G-banding were carried out using the methods of Sumner (1972) and Cano et al. (1996), respectively. Restriction endonucleases were used according to Sánchez et al. (1990) with some modifications, i.e., *Alu*I (0.3 U/µL at 37°C for 4 h), *Bam*HI (0.3 U/µL at 37°C for 14 h) and *Eco*RI (2.0 U/µL at 37°C for 14 h).

Figure 1. Specimen of *Steindachneridion scripta* from Paranapanema River. Total length ~80 cm.

RESULTS

The modal diploid number observed in *S. scripta* examined was 56 chromosomes, with no chromosomal differences detected between sexes. The chromosome formula established consisted of 24 metacentrics, 20 submetacentrics, 4 subtelocentrics, and 8 acrocentrics, and the fundamental number was 104 (Figure 2). A consistently observed characteristic was the presence of a secondary constriction on the short arm of pair 25.

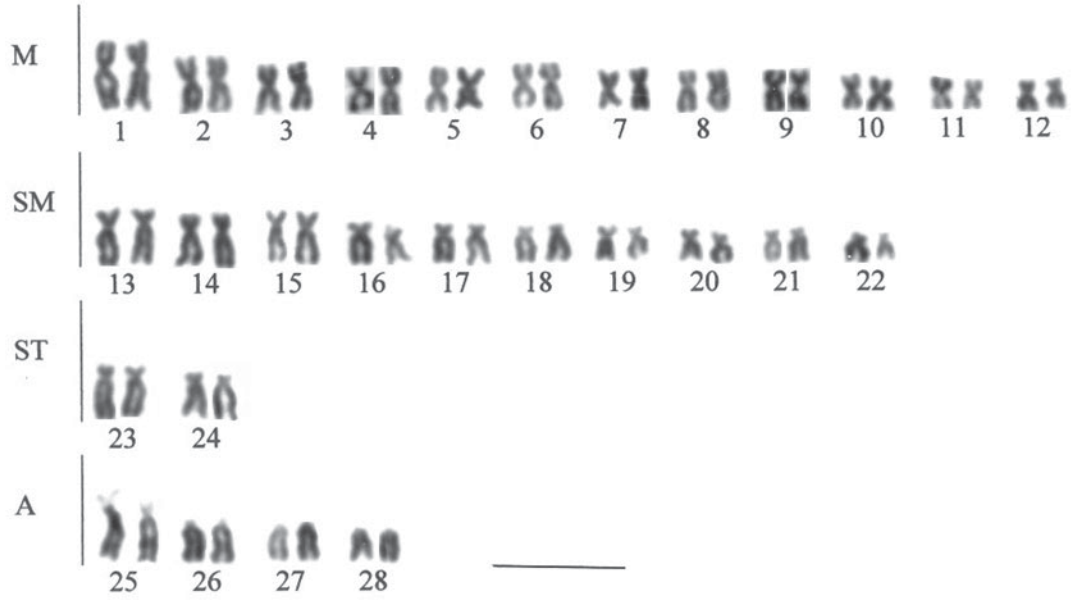

Figure 2. Giemsa-stained karyotype of *Steindachneridion scripta*. Scale bar = 5 µm. M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric.

Heterochromatin was visualized by means of C-banding in telomeric regions of several chromosomes of the complement (Figure 3a). Some metacentric chromosomes showed conspicuous bands in both telomeres, and the secondary constriction of the first acrocentric pair (No. 25) was clearly heterochromatic. The bands were found on the entire short arms and the pericentromeric region of the subtelocentric and acrocentric chromosomes (Figure 3a).

Chromosome preparations of *S. scripta* were treated with the restriction enzyme *Alu*I, producing a C-banding-like pattern in a few chromosome pairs (pairs 23 and 24). However, several C-band negative centromeric regions were alternatively cleaved by this enzyme (Figure 3b).

G-banding produced a staining pattern that allowed the identification of many homologue chromosomes. The secondary constriction (in pair 25) was G-banding negative (Figure 4a). Treatment with *Bam*HI revealed dark bands on some chromosomes of the complement, generating a G-banding-like staining (Figure 4b). *Eco*RI produced characteristic bands, with target sites distributed principally in centromeric regions of metacentric chromosomes, which consequently showed a pale staining when digested (Figure 5).

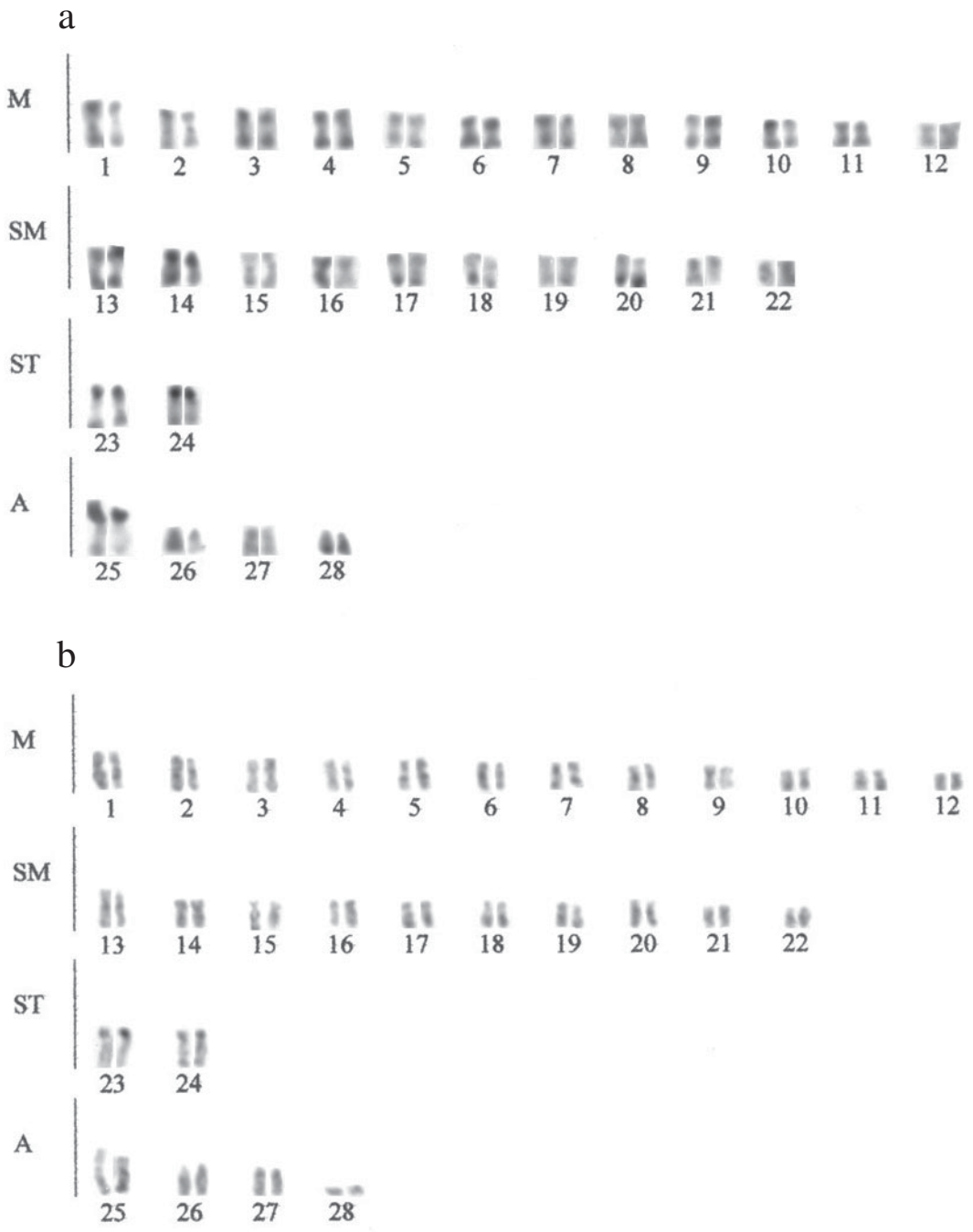

Figure 3. Differentially stained karyotypes of *Steindachneridion scripta*. **a.** C-banded and **b.** *Alu*I-treated. For abbreviations, see legend to Figure 2.

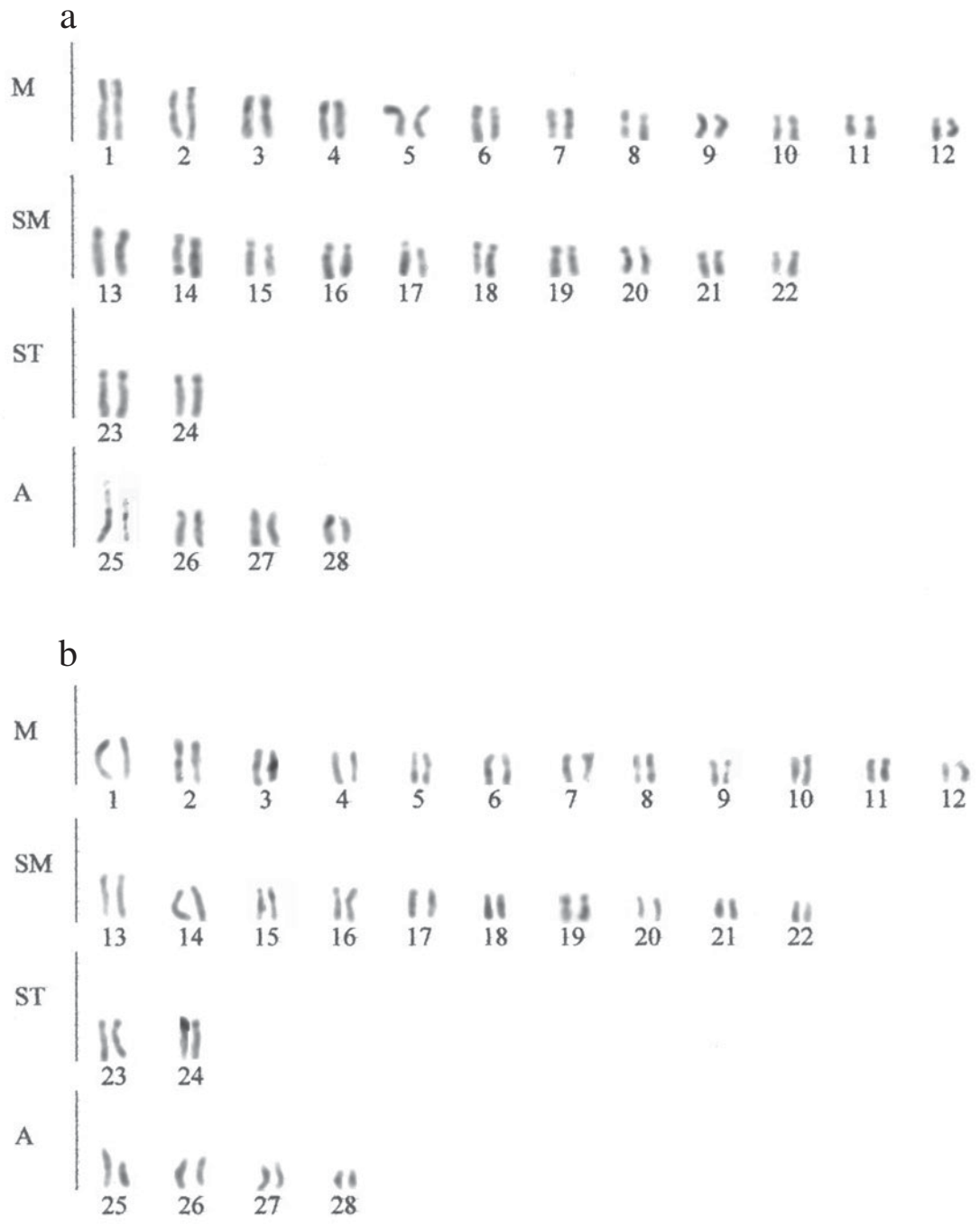

Figure 4. Differentially stained karyotypes of *Steindachneridion scripta*. **a.** G-banded and **b.** *Bam*HI-treated. For abbreviations, see legend to Figure 2.

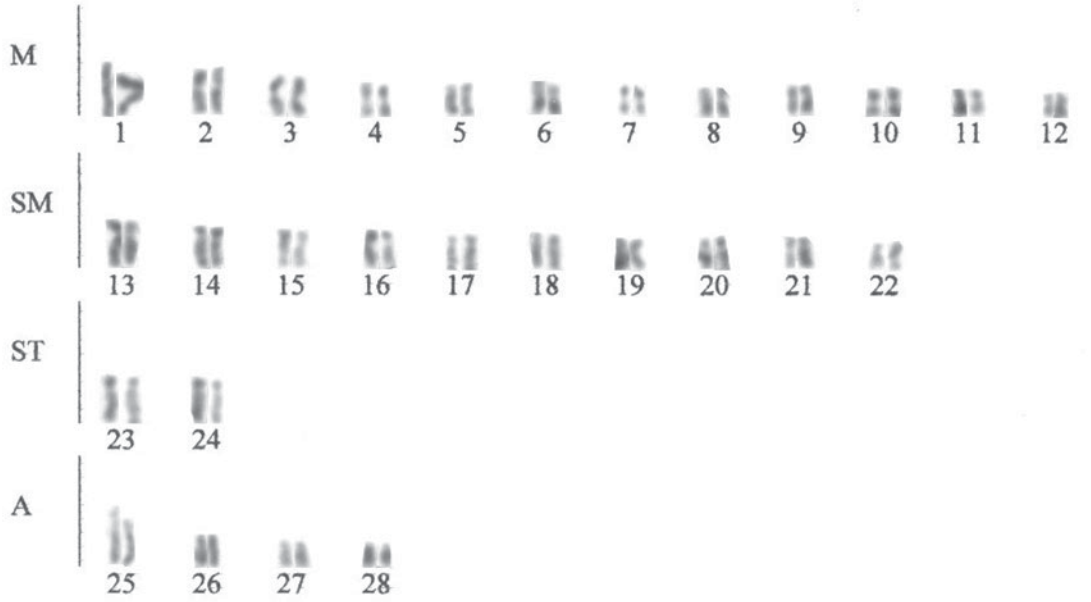

Figure 5. Karyotype of *Steindachneridion scripta* treated with *Eco*RI. For abbreviations, see legend to Figure 2.

DISCUSSION

A karyotype of 56 chromosomes with a high number of bi-armed elements constitutes the most frequent cytogenetic feature in Pimelodidae, particularly in species of Sorubiminae (Fenocchio and Bertollo, 1992; Martins-Santos et al., 1996; Swarça et al., 2001), as well as in *S. scripta* (Figure 2). Our analysis of this karyotype showed that the first pair of metacentric chromosomes is nearly 30% larger than the second, with the rest being gradually smaller in size. This characteristic could help in comparative studies with other species of the genus.

On the other hand, the secondary constrictions on the short arm of the first pair of acrocentrics, shown to be associated with nucleolar organizer regions (Swarça, 2003), when stained conventionally, appeared consistently decondensed and heteromorphic (Figure 2). These also represent common features in other pimelodid species, which show nucleolar organizer regions on secondary constrictions of short chromosome arms, as has been reported in *Pseudoplatystoma*, *Rhamdia* and *Zungaro* species (Fenocchio and Bertollo, 1992; Swarça et al., 2001; Fenocchio et al., 2003).

Obtaining multiple bands (G or R) along chromosomes is a difficult task in fish cytogenetics, and few studies of Neotropical species using this approach have been reported. This applies, for instance, to characterizing and investigating the origin of a multiple sex chromosome system (Bertollo et al., 1997), to comparing different staining methods and banding techniques (Maistro et al., 1999), and to characterizing A and B chromosomes (Maistro et al., 2000). We were able to obtain good multiple banding patterns in *S. scripta* with several techniques (C-, G- and restriction enzyme banding), allowing the identification of several homologues in the chromosome complement.

The C-banding pattern observed, with bands distributed in distal and telomeric regions of chromosome arms, sometimes covering the entire short arm, resembles the pattern observed in other species of this family (Fenocchio et al., 2000; Abucarma and Martins-Santos, 2001), suggesting an equilocal distribution of heterochromatin. The bi-armed pairs, showing bands on both telomeres (i.e., 1, 3, 4, 6, 7, 9, 13), and the subtelocentric ones (pairs 23 and 24), with bands occurring on short arms, could be good chromosome markers. Secondary constrictions showed strongly heterochromatic bands (pair 25, Figure 3a). This feature seems to be common in pimelodids and has been observed in *Zungaro zungaro* (Swarça et al., 2001) and in *Steindachneridion* and *Rhamdia* (Swarça et al., 2003).

It has been reported that *Alu*I banding of fish chromosomes produces a C-banding-like pattern (Maistro et al., 1999, 2000); however, we found that several chromosome pairs in *S. scripta* had target sites coinciding with C-bands, producing a reverse pattern, as could be seen in some metacentric elements, as well in secondary constrictions. However, the C-band positive regions on the short arms of pairs 23 and 24 clearly had no target sites for this enzyme, staining darkly (Figure 3b).

G-banding and *Bam*HI treatment seems to produce the same staining pattern, as has been suggested by Maistro et al. (1999, 2000). These techniques allowed the pairing of several chromosomes of the complement; otherwise, it is nearly impossible to recognize the correct pairing (Figure 4a,b). The use of *Eco*RI indicated target sites at centromeres and pericentromeric regions of several metacentric and submetacentric pairs, displaying a final staining pattern that was similar, but not identical, to that made with other enzymes (Figure 5).

With the application of restriction enzymes, principally *Alu*I, centromeric and pericentromeric regions showed different base compositions among the chromosomes, where they were found to be differentially cleaved by each enzyme, indicating more than one kind of heterochromatin. Furthermore, the short arms of pairs 23 and 24 were not cleaved, showing normal Giemsa staining; however, the secondary constriction of pair 25 always appeared pale when treated with trypsin (G-banding) and restriction enzymes (Figures 3b; 4a,b; 5), probably because this region is comprised of a series of different repeated target sequences.

The present results provided an integral karyotypic characterization of *S. scripta*; several chromosomes could be considered cytogenetic markers. These chromosome markers could be helpful tools in future basic and applied studies, not only within the genus *Steindachneridion*, but also among other genera of Sorubiminae.

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REFERENCES

Abucarma M and Martins-Santos IC (2001). Karyotype and B chromosomes of *Rhamdia* species (Pisces, Pimelodidae) endemic in the River Iguaçu Basin. *Cytologia* 66: 299-306.

Bertollo LAC, Fontes MS, Fenocchio AS and Cano J (1997). The X_1X_2Y sex chromosome system in the fish *Hoplias malabaricus*. I. G-, C- and chromosome replication banding. *Chromosome Res.* 5: 493-494.

Burguess WE (1989). An atlas of freshwater and marine catfishes. TFH Publications Inc., Neptune City, NJ, USA.

Cano J, Pretel A, Melendez S, Garcia F et al. (1996). Determination of early stages of sex chromosome differentiation in the sea bass *Dicentrarchus labrax* L. (Pisces, Perciformes). *Cytobios* 87: 45-59.

Fenocchio AS and Bertollo LAC (1988). A simple method for fresh-water fish lymphocyte culture. *Rev. Bras. Genet.* 11: 847-852.

Fenocchio AS and Bertollo LAC (1992). Karyotype similarities among Pimelodidae (Pisces, Siluriformes) from the Brazilian Amazon region. *Cytobios* 69: 41-46.

Fenocchio AS, Bertolo LAC, Takahashi CS and Camacho JPM (2000). B chromosomes in two fish species, genus *Rhamdia* (Siluriformes, Pimelodidae). *Folia Biol.* 48: 105-109.

Fenocchio AS, Swarça AC, Cestari MM and Dias AL (2003). Karyotypic characterization and NOR analysis by different banding techniques of *Rhamdia quelen* (Pisces, Pimelodidae) from the First Plateau of the Iguaçu River (Brazil). *Folia Biol.* 51: 3-4.

Lundberg JG and Littmann MW (2003). Pimelodidae (long-whiskered catfishes). In: Checklist of the freshwater fishes of South and Central America (Reis RE, Kullander SO and Ferraris Jr CJ, eds.). EDIPUCRS, Porto Alegre, RS, Brazil, pp. 432-446.

Maistro EL, Foresti F and Oliveira C (1999). R- and G-band patterns in *Astyanax scabripinnis* (Pisces, Characiformes, Characidae). *Genet. Mol. Biol.* 22: 201-204.

Maistro EL, Oliveira C and Foresti F (2000). Cytogenetic analysis of A- and B-chromosomes of *Prochilodus lineatus* (Teleostei, Prochilodontidae) using different restriction enzyme banding and staining methods. *Genetica* 108: 119-125.

Martins-Santos IC, Julio Jr HF and Burin I (1996). Karyotypic studies of four species of the Sorubiminae subfamily (Pisces, Siluriformes). *Caryologia* 49: 73-80.

Sanchez L, Martinez P, Vinas A and Bouza C (1990). Analysis of the structure and variability of nucleolar organizer regions of *Salmo trutta* by C-, Ag-, and restriction endonuclease banding. *Cytogenet. Cell Genet.* 54: 6-9.

Sumner AT (1972). A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* 75: 304-306.

Swarça AC (2003). Contribuição à citogenética dos Pimelodidae de grande porte: estudos cariotípicos de 4 espécies do "subgrupo" Sorubiminae. Doctoral thesis, Departamento de Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, PR, Brazil.

Swarça AC, Cestari MM, Giuliano-Caetano L and Dias AL (2001). Cytogenetic characterization of the large South American siluriform fish species *Zungaro zungaro* (Pisces, Pimelodidae). *Chromosome Sci.* 5: 51-55.

Swarça AC, Fenocchio AS, Cestari MM and Dias AL (2003). Analysis of heterochromatin by combination of C-banding and CMA_3 and DAPI staining in two fish species (Pimelodidae, Siluriformes). *Genetica* 119: 87-92.