

## Banding Chromosome Pattern of Two Species of *Pimelodus* (Siluriformes, Pimelodidae) from the Parana River Basin of Brazil

Lenice de SOUZA, Lucia GIULIANO-CAETANO and Ana Lúcia DIAS

Accepted September 6, 2004

SOUZA L. de, GIULIANO-CAETANO, L., DIAS A. L. 2004. Banding chromosome pattern of two species of *Pimelodus* (Siluriformes, Pimelodidae) from the Parana River basin of Brazil. Folia biol. (Kraków) 52: 165-169.

Cytogenetic studies were carried out on seven specimens of *Pimelodus heraldoi* and sixteen specimens of *Pimelodus* sp., both from the Parana River basin. The two species had the same diploid number of 56 chromosomes: *P. heraldoi* with 22M+22SM+6ST+6A and FN of 106 and *Pimelodus* sp. with 24M+26SM+4ST+2A and FN of 110. NORs were found at the terminal position of the long arm of one pair of ST chromosomes. C-banding (CB) showed in the two species heterochromatin distributed in various chromosomes of the complement, mainly in telomeric regions and in a pair of metacentric chromosomes with strong heterochromatic staining in both telomeres. Treatment only with the fluorochrome CMA<sub>3</sub> confirmed in *Pimelodus heraldoi* and *Pimelodus* sp. the nucleolar chromosome pair and showed other fluorescent bands. Combined treatment with CB+CMA<sub>3</sub> enhanced fluorescent staining of chromosomes in the two fish species evidencing several bands, including in *P. heraldoi* a chromosome pair showing fluorescent staining in both telomeres.

Key words: Karyotype, heterochromatin, Ag-NOR, CMA<sub>3</sub>, *Pimelodus*, Siluriformes.

Lenice de SOUZA, Lucia GIULIANO-CAETANO, Ana L. DIAS, UEL, Dept., Biol. Geral, CCB, CEP86051-970, Londrina, PR, Brazil.  
E-mail: lenicesouza@hotmail.com  
giuliano@uel.br; anadias@uel.br

There are 54 karyotyped species in the family Pimelodidae, 23 of which have a karyotype considered to be standard for this group,  $2n=56$  (OLIVEIRA & GOSZTONYI 2000). Nonetheless, this family displays an extensive variation in karyotype, from  $2n=46$  observed in *Pimelodella* sp. (DIAS & FORESTI 1993) to  $2n=63$  found in *Rhamdia hilarii* (FENOCCHIO & BERTOLLO 1990), taking into account extra chromosomes, which indicates that various types of chromosomal rearrangements must be involved in the process of speciation in this group, as has been suggested by OLIVEIRA *et al.* (1988).

According SHIBATTA (2003) the systematics of the very specious family Pimelodidae is really complex, appearing, consequently, many nomenclatural problems. In isolate rivers it is possible to find endemic unnamed species, as is the case of *Pimelodus* sp. from the Iguaçú river (GARAVELLO *et al.* 1997).

The genus *Pimelodus*, considered the group containing the most species within the family Pimelodidae, has 27 species distributed throughout the hydrographic basins of South America, and there are only few cytogenetic data on approximately 11 species (SWARÇA *et al.* 2001; BORIN & MARTINS-SANTOS 2002).

Results of chromosomal banding, such as C-banding, NOR and chromomycin A<sub>3</sub> (CMA<sub>3</sub>), have been reported for the genus *Pimelodus* by various authors, such as VISSOTTO *et al.* (1999); SWARÇA *et al.* (2001) and BORIN & MARTINS-SANTOS (2002). Available data show heterochromatin distributed preferentially in centromeric and/or telomeric regions, and treatment with CMA<sub>3</sub> generally has shown fluorescent staining in correspondence with the nucleolar chromosomes.

In the present study, a cytogenetic investigation of two species of fish belonging to the genus *Pimelodus* was conducted to determine their karyotype and band patterns. The conventional C-banding technique (CB) and treatment with the fluorochrome CMA<sub>3</sub> were employed, separately and consecutively, to contribute to a better understanding of the distribution and composition of heterochromatin.

### Material and Methods

Seven specimens (1 male and 6 females) of *Pimelodus heraldoi* collected from the Tibagi River,

Parana, Brazil and sixteen specimens (6 males and 10 females) of *Pimelodus* sp., endemic undescribed species from the Iguaçu River, Parana, Brazil, were analysed in the present study.

Mitotic chromosome preparations were obtained from lymphocyte culture in *Pimelodus* sp. according to FENOCCHIO and BERTOLLO (1988) and from kidney cells in *Pimelodus heraldoi* by the direct method according BERTOLLO *et al.* (1978). Chromosome morphology was determined on the basis of arm ratio as proposed by LEVAN *et al.* (1964). The fundamental number (FN) was determined considering metacentric (M), submetacentric (SM) and subtelocentric chromosomes (ST) as biarmed. Acrocentric (A) were considered as un-armed. NOR silver staining was performed using the method of HOWELL and BLACK (1980). C-banding (CBG) were obtained using the method described by SUMNER (1972) and chromomycin (CMA<sub>3</sub>) staining as described by SCHMID (1980). A set of chromosome preparations were pretreated with C-banding (CB) and stained in the dark with CMA<sub>3</sub>.

## Results

The two species of *Pimelodus* investigated in the present study were shown to have the same diploid number of 56 chromosomes. However, they differed in karyotypic formulae with high fundamental numbers (FN). The karyotype for *P. heraldoi* was 22M+22SM+6ST+6A with FN of 106 (Fig. 1) and for *Pimelodus* sp., 24M+26SM+4ST+2A with FN of 110 (Fig. 2).

In the two species examined, the Ag-NOR was observed at the terminal position of the long arm of a pair of subtelocentric chromosomes, pair 25 in *P. heraldoi* and pair 26 in *Pimelodus* sp. (Figs 1, 2), being observed in some metaphase plates of *Pimelodus* sp. a NOR size heteromorphism (Fig. 2).

By use of the C-banding technique with Giemsa staining (CBG) heterochromatic regions were found to be distributed in several chromosomes of the complement, mainly in the telomeric regions. Almost all showed weak staining in both telomeres, while there was evidence of one pair of metacentric chromosomes with strong heterochromatic staining in both telomeres, this being a marker pair for these two species (Figs 3A, B). It is interesting to note that one of the homologue chromosomes of this marker pair, stains stronger in *P. heraldoi* (Figs 3A).

Treatment only with the fluorochrome CMA<sub>3</sub> confirmed the NORs localization and showed in *Pimelodus heraldoi* fluorescent staining of other chromosomes of the complement, at telomeric locations, with one chromosome showing evidence

of staining in both telomeres (Fig. 3C), probably the same shown by C-banding. However, in *Pimelodus* sp. was stained the NOR-bearing pair and an additional metacentric chromosome pair with weak telomere fluorescence (Fig. 3D).

When the chromosomes of the two species of *Pimelodus* studied here were first C-banded and then stained with CMA<sub>3</sub> (CB+CMA<sub>3</sub>), many fluorescent signals appeared, being more evident in *Pimelodus heraldoi* mainly the chromosome pair with both telomeres stained (Fig. 3E). In *Pimelodus* sp., besides the NOR-bearing chromosome pair, there was also observed additional fluorescent signals, including a chromosome pair with both telomeres stained (Fig. 3F), a staining pattern which was not evidenced with CMA<sub>3</sub> treatment alone.

## Discussion

Of the 27 species of *Pimelodus* described to date, 11 have been karyotyped with a diploid number of 56, which is highly conservative for this group of fish (VISSOTTO *et al.* 1999; SWARÇA *et al.* 2001; BORIN & MARTINS-SANTOS 2002), and adding to this number are the two species studied here. Considering that 2n= 56 is the base number for Siluriformes, according to OLIVEIRA & GOSZTONYI (2000), and that almost all fish in this group have this modal number, it appears that the genus *Pimelodus* conforms with this plesiomorphic characteristic (primitive trait).

Although the diploid number in the majority of species in this group of fish is conservative, variation in the karyotypic formulae has been frequently observed, as demonstrated in *Pimelodus heraldoi* and *Pimelodus* sp. of the Parana River basin, as well as in various species of the genus summarized by SWARÇA *et al.* (2000). This diversification in karyotype could be related to chromosomal rearrangements, involving inversions and/or translocations having occurred during the evolutionary processes of this group. A high fundamental number is an outstanding characteristic in the genus *Pimelodus*, due to the large number of biarmed chromosomes, varying from 106 in *P. maculatus* from the Tibagi River (SWARÇA *et al.* 2001) to 110 in *Pimelodus* sp. from the Iguaçu River (present study).

The distribution of constitutive heterochromatin at the centromeric and/or telomeric position, is the situation most frequently observed in different species of *Pimelodus*, such as *Pimelodus absconditus* and *P. ornatus* (BORIN & MARTINS-SANTOS 2002), as well in *Pimelodus maculatus* (VISSOTTO *et al.* 1999; SWARÇA *et al.* 2001; BORIN & MARTINS-SANTOS 2002).

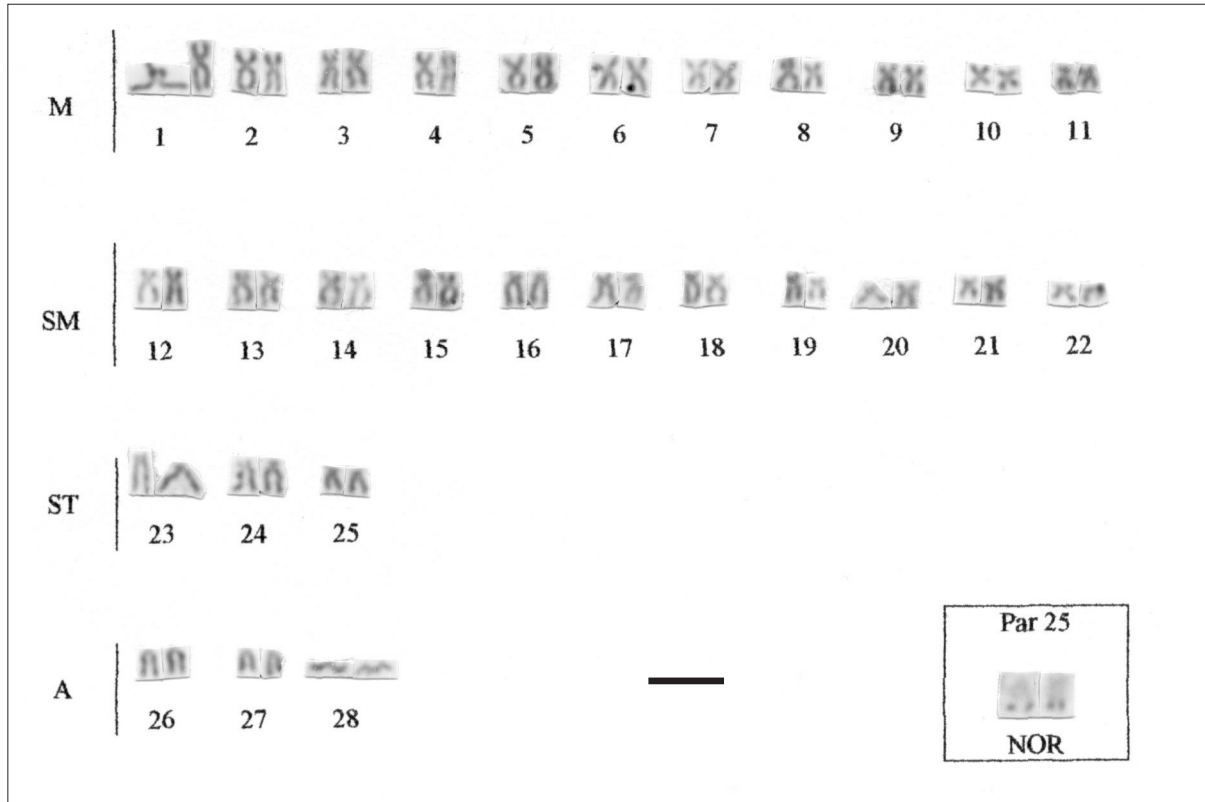


Fig. 1. Giemsa-stained karyotype of *Pimelodus heraldoi*. The inset shows the pair 25 with Ag-NOR stained terminal region. Bar = 5  $\mu$ m.

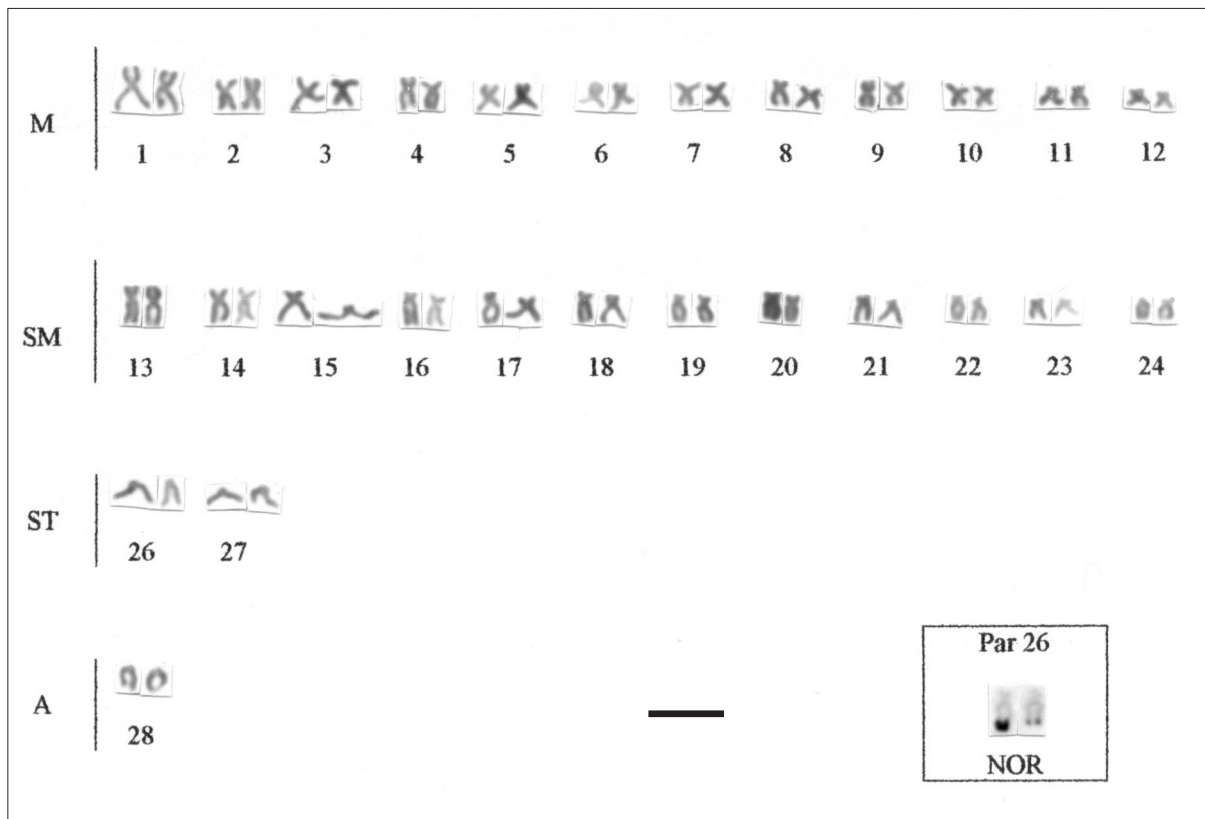


Fig. 2. Giemsa-stained karyotype of *Pimelodus* sp. The inset shows the pair 26 with Ag-NOR stained terminal region. Bar = 5  $\mu$ m.

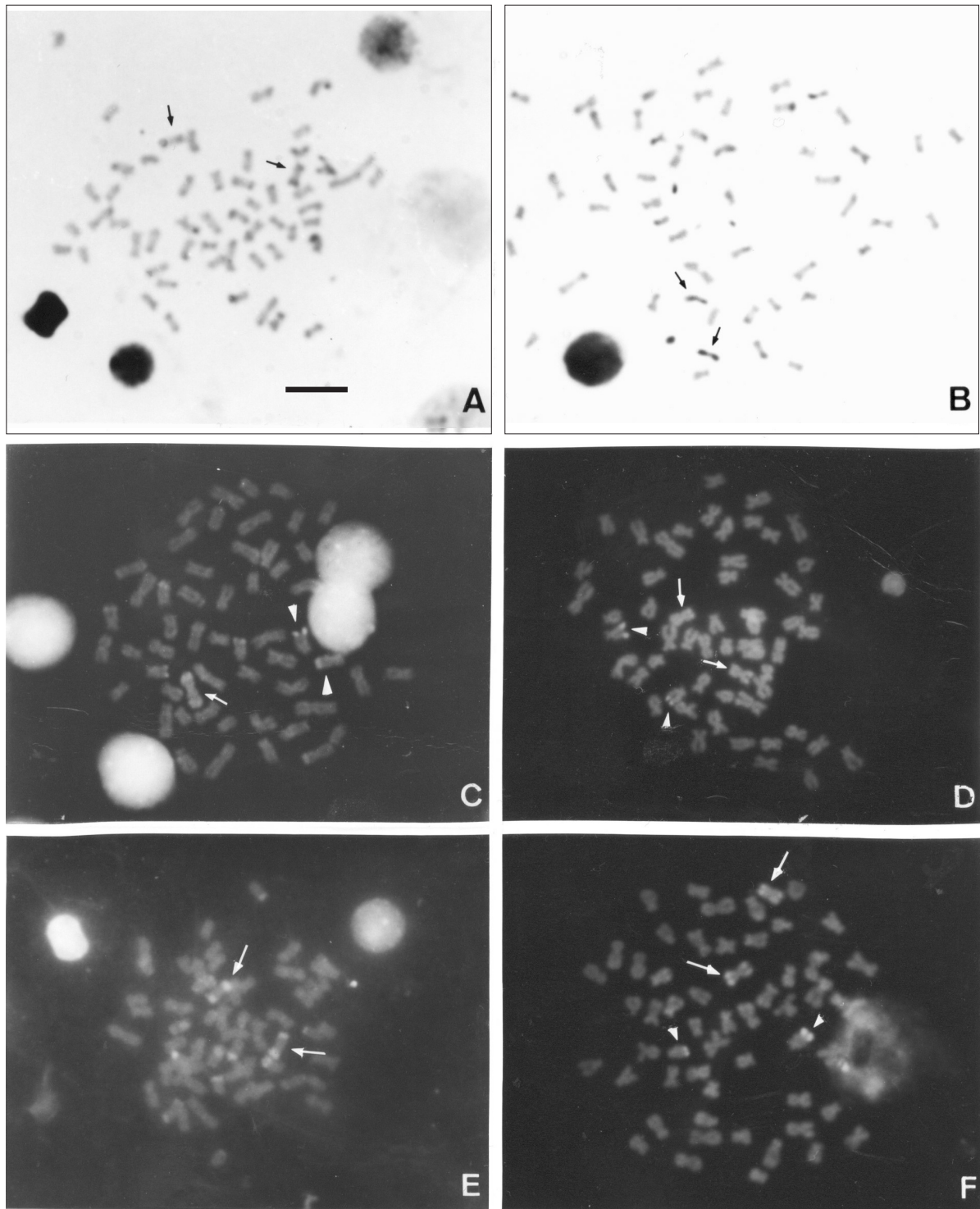


Fig. 3. CBG, CMA<sub>3</sub> and CB plus CMA<sub>3</sub> staining of *Pimelodus heraldoi* (A, C, E) and *Pimelodus* sp. (B, D, F), respectively. The arrows in (A) and (B) show one chromosome pair with bitelomeric CB staining; in (C), (D) and (F) the arrowheads show the NOR-bearing chromosome pair positive for CMA<sub>3</sub> staining; the arrows show in (C), (E) and (F) the chromosomes with bitelomeric fluorescent staining; in (D) the arrows show one chromosome pair positive for CMA<sub>3</sub> staining. Bar = 5  $\mu$ m.

The presence of the metacentric pair with conspicuous heterochromatic staining of both telomeres appears to be a marker trait of some species of the family Pimelodidae, for example, *Pimelodella* aff. *avanhandavae* (SWARÇA *et al.* 2003a), *Iheringichthys labrosus* (CARVALHO 2001) and *Pimelodella* sp. (VASCONCELOS & MARTINS-

-SANTOS 2000), among others, suggesting that the occurrence of this chromosome marker in the family Pimelodidae, including the two species of *Pimelodus* studied here, can be an important tool in the phylogenetic understanding of this group of fish. According to MARGARIDO and GALETTI Jr. (1999), the distribution pattern of heterochromatin

permits the establishment of such relationships, including the characterization and differentiation of some species of fish, which would help in delineating the mechanisms occurring during the karyotype evolution of these organisms.

Based on the results with CMA<sub>3</sub> treatment, and considering that almost all telomeres in the two species were C-band positive, it is evident that some heterochromatic telomeric regions, mainly in *P. heraldoi*, are CMA<sub>3</sub> positive. This includes the NOR-bearing chromosome pair, since this region is associated with DNA families rich in GC bases in almost all the fish groups (Gold *et al.* 1990). Single NORs at the terminal position of long arms is very frequent in the genus *Pimelodus*, although variations in the chromosome type and localization of this region can occur (SWARÇA *et al.* 2001; BORIN & MARTINS-SANTOS 2002).

Pretreatment with C-banding is believed to relax DNA and increase the accessibility of the fluorochromes (SWARÇA *et al.* 2003b), allowing for a more detailed analysis of the composition of heterochromatin and making it possible to gain greater insight into the chromosomal constitution of fish. Therefore, present results indicate that in the two species of *Pimelodus*, there are some GC-rich heterochromatic regions that are visualized only after treatment with CB+CMA<sub>3</sub>. In *Pimelodus heraldoi*, the pair showing staining in both telomeres, which was heterochromatic, was much more evident, as were various other telomeric regions. In *Pimelodus* sp. the pair showing bitelomeric fluorescent signals, which was not visualized only with CMA<sub>3</sub> staining, could also be seen, just as other chromosomes; however, differently from *P. heraldoi*, telomere bands were weak.

Comparing the results obtained from these combined treatments, the differentiation in the composition and distribution of the GC base pairs in the chromosome complement of *Pimelodus* sp. and *P. heraldoi* is evident. Thus, these techniques when used in combination provide an important tool for a better understanding of chromosome structure and for cytogenetic comparisons among different species of fish.

It should be pointed out that the present study provides an important contribution to the cytogenetics of the genus *Pimelodus*, since these findings are the first cytogenetic data on *P. heraldoi* and *Pimelodus* sp., whereby according to GARAVELLO *et al.* (1997) the latter species is endemic to the Iguaçú River and is being described.

## Acknowledgements

The authors are grateful to CAPES for their financial support. We are also thankful to Dr. O. A. SHIBATTA by identifying of the species analysed and to Dr. A. S. FENOCCHIO for a critical reading of the manuscript.

## References

- BERTOLLO L. A. C., TAKAHASHI C. S., MOREIRA-FILHO O. 1978. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). *Rev. Brasil. Genet.* **1**: 103-120.
- BORIN L. A., MARTINS-SANTOS I. C. 2002. Cytogenetic aspects in species of the genus *Pimelodus* (Pisces, Siluriformes, Pimelodidae) of the River Paraná Basin. *Cytologia* **67**: 199-204.
- CARVALHO R. A. 2001. Análise citogenética em uma população de *Iheringichthys labrosus* (Pisces, Pimelodidae), com ênfase à ocorrência de cromossomos supranumerários. Monografia. Universidade Estadual de Londrina, Londrina. PR.
- DIAS A. L., FORESTI F. 1993. Cytogenetic studies on fishes of the family Pimelodidae (Siluroidei). *Rev. Brasil. Genet.* **16**: 585-600.
- FENOCCHIO A. S., BERTOLLO L. A. C. 1988. A simple method for fresh-water fish lymphocyte culture. *Rev. Brasil. Genet.* **11**: 847-852.
- FENOCCHIO A. S., BERTOLLO L. A. C. 1990. Supernumerary chromosome in a *Rhamdia hilarii* population (Pisces, Pimelodidae). *Genetica* **81**: 193-198.
- GARAVELLO J. C., PAVANELLI C. S., SUZUKI H. I. 1997. Caracterização da ictiofauna do rio Iguaçú, (In: A. A. Agostinho & L. C. Gomes, Reservatório de Segredo: bases ecológicas para o manejo. Maringá, EDUEM): 61-84.
- GOLD J. R., LI Y. C., SHIPLEY N. S., POWERS P. K. 1990. Improved methods for working with fish chromosomes with a review of metaphase chromosome banding. *J. Fish Biol.* **37**: 563-575.
- HOWELL W. M., BLACK D. A. 1980. Controlled silver staining of nucleolus organizing regions with a protective colloidal developer: a one step method. *Experientia* **36**: 1014-1015.
- LEVAN A., FREDGA K., SANDBERG A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* **52**: 201-220.
- MARGARIDO V. P., GALETTI Jr. P. M. 1999. Heterochromatin patterns and karyotype relationships within and between the genera *Brycon* and *Salminus* (Pisces, Characidae). *Genet. Mol. Biol.* **22**: 357-362.
- OLIVEIRA C., GOSZTONYI A. E. 2000. A cytogenetic study of *Diplomystes mesembrinus* (Teleostei, Siluriformes, Diplomystidae) with a discussion of chromosome evolution in Siluriformes. *Caryologia* **53**: 31-37.
- OLIVEIRA C., ALMEIDA-TOLEDO L. F., FORESTI F., BRITSKI H., TOLEDO-FILHO S. 1988. Chromosome formulae of Neotropical freshwater fishes. *Rev. Bras. Genet.* **11**: 577-624.
- SCHMID M. 1980. Chromosome banding in Amphibia, IV. Differentiation of GC – and AT – rich chromosome regions in Anura. *Chromosoma* **77**: 83-103.
- SHIBATTA O. 2003. Phylogeny and Classification of Pimelodidae, (In: G. Arratia, B.G. Kapoor, M. Chardon & Rui Diogo, Catfishes. Science Publishers, Inc. Enfield, USA, Plymouth, UK): 385-400.
- SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell Res.* **75**: 304-306.
- SWARÇA A. C., GIULIANO-CAETANO L., DIAS A. L. 2000. Cytogenetics of species of the families Pimelodidae and Rhamdiidae (Siluriformes). *Genet. Mol. Biol.* **23**: 589-593.
- SWARÇA A. C., GIULIANO-CAETANO L., DIAS A. L. 2001. Analyses of nucleolus organizer regions and heterochromatin of *Pimelodus maculatus* (Pisces, Pimelodidae). *Genetica* **110**: 97-100.
- SWARÇA A. C., VIDOTTO A. P., DIAS A. L. 2003a. Cytogenetic characterization of *Pimelodella* aff. *avanhandavae* (Siluriformes, Pimelodidae) from Tibagi river (Parana State, Brazil). *Caryologia* **56**: 421-425.
- SWARÇA A. C., FENOCCHIO A. S., CESTARI M. M., DIAS A. L. 2003b. Analysis of heterochromatin by combination of C-banding and CMA<sub>3</sub> and DAPI staining in two fish species (Pimelodidae, Siluriformes). *Genetica* **119**: 87-92.
- VASCONCELOS C., MARTINS-SANTOS I. C. 2000. Chromosome polymorphism in species of the Pimelodidae family (Pisces, Siluriformes). *Hereditas* **132**: 103-109.
- VISSOTTO P. C., FORESTI F., OLIVEIRA C. 1999. Karyotype description of five species of Pimelodidae (Teleostei, Siluriformes). *Chromosome Science* **3**: 1-7.