

# Comparative chromosomal analyses in species of the genus *Pimelodella* (Siluriformes, Heptapteridae): occurrence of structural and numerical polymorphisms

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**Abstract** — Cytogenetic analyses were carried out in five species of *Pimelodella* from the main sub-basins of Upper Paraná River and Paraíba do Sul River. The diploid number ranged from  $2n = 46$  to  $2n = 58$  chromosomes, and all populations differed in the karyotype constitution. The presence of supernumerary chromosomes as well as the occurrence of a XX/XY sex chromosome system and heterochromatin polymorphisms were detected. The 18S rDNA FISH confirmed the presence of single NORs and revealed additional sites on supernumerary chromosomes. The number and location of 5S rDNA sites were variable. Aspects related to the karyotypic evolution within the genus are discussed.

**Key words:** *Pimelodella*, polymorphism, sex chromosomes, supernumerary chromosomes, XX/XY.

## INTRODUCTION

Siluriform fishes are widespread throughout nearly all regions on the planet, excepting the poles, including marine, estuarine and freshwater forms (GRANDE and EASTMAN 1986). Over the Neotropical region, the order Siluriformes is the second largest group in number of species and diversity, right after Characiformes (NELSON 2006). Most representatives of this order inhabit freshwater environments and present a predominantly nocturnal activity (MEES 1974).

The family Heptapteridae comprises small to medium-sized fish and represents one of the widest radiations of Neotropical catfishes (DE PINNA 1998). It is composed of 25 genera, where the genus *Pimelodella*, popularly known in Brazil as “mandi-chorão” stands out in diversity. The major

morphological feature of this genus is the presence of a dark horizontal line that extends from the nose up to the base of the caudal peduncle.

Cytogenetic studies in heptapterids indicated that *Pimelodella* can serve as an interesting material, once different diploid numbers (46, 52 and 58 chromosomes) and distinct karyotype compositions have been reported in the populations analyzed so far (SWARÇA *et al.*, 2000), along with structural (VASCONCELOS and MARTINS SANTOS 2000) and numerical polymorphisms (ALMEIDA-TOLEDO *et al.* 1992), besides the occurrence of the only case of sex chromosomes within the family Heptapteridae (DIAS and FORESTI 1993). However, because of taxonomical constraints leading to misidentification of species, some cytogenetic features might have been attributed to ambiguous species (VIDOTTO *et al.* 2004).

The goal of the present study was to characterize, by molecular and classic cytogenetic techniques, different species and populations of *Pimelodella* from distinct Brazilian basins in order to obtain new information about their karyotypic features and to evaluate the chromosomal evolutionary trends within the genus.

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## MATERIAL AND METHODS

Five species of *Pimelodella* from distinct hydrographic basins of the states of São Paulo and Rio de Janeiro, Brazil, were cytogenetically analyzed: *P. boschmai*, *P. meeki*, *P. lateristriga*, *P. gracialis* and *Pimelodella* sp (Table 1). The specimens were identified in the Zoology Museum of the Universidade de São Paulo (MUZUSP) and deposited in the Ichthyogenetics Laboratory of the Universidade de São Paulo.

The mitotic chromosomal preparations were obtained from kidney cells using the procedure adapted by GOLD *et al.* (1990) for fish studies. The chromosomal morphology was determined based on arm ratio as proposed by LEVAN *et al.* (1964). The fundamental number (FN) was calculated taking into account that metacentric (m), submetacentric (sm) and subtelocentric (st) chromosomes are bi-armed and acrocentric (a) chromosomes bear a single arm.

The characterization of active nucleolar organizer regions (NORs) was performed according to HOWELL and BLACK (1980). The GC-specific fluorochrome staining followed the technique by SCHMID (1980) with slight modifications, using chromomycin A<sub>3</sub> (CMA<sub>3</sub>). The blocks of constitutive heterochromatin were detected according to the C-banding procedure described by SUMNER (1972). Fluorescent *in situ* hybridization (FISH) followed PINKEL *et al.* (1986) using 18S rDNA probes (HATANAKA and GALETTI JR., 2004) and 5S rDNA probes (MARTINS and GAL-LETTI JR., 1999).

## RESULTS

In the present work approximately 50 metaphases were analysed per individual. The diploid number found in the studied populations ranged from 46 to 58 chromosomes. The populations with  $2n = 46$  chromosomes were also differentiated by their karyotype formulae (Fig. 1a-e) (Table 1). In *P. boschmai*, although males and females share the same chromosomal formula, there is a difference in the karyotype related to the presence of a single sex chromosomal system of XX/XY type, where the X is a small metacentric and the Y is a medium metacentric element. The sex chromosomes correspond to the 19<sup>th</sup> pair (Fig. 1a).

An interindividual numerical variation was found in the diploid number of *Pimelodella* sp. due to the presence of up to four small super-

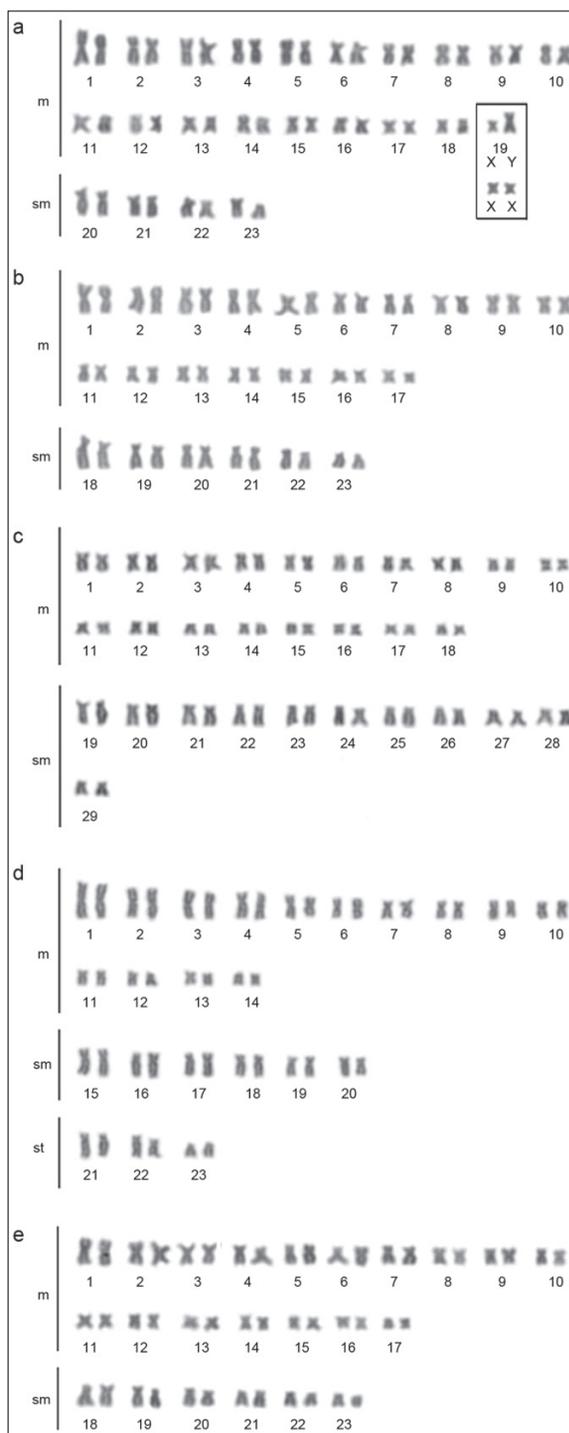


Fig. 1 — Karyotype of (a) *P. boschmai*; (b) *P. gracialis*; (c) *P. lateristriga*; (d) *P. meeki* and (e) *Pimelodella* sp. after conventional Giemsa staining. In detail, the sex chromosomal pair of *P. boschmai*.

numerary chromosomes (found in a single individual) (Fig. 1e).

All the species presented NORs located on short arms of a pair of submetacentric chromo-

TABLE 1 — Cytogenetic data of the *Pimelodella* species analyzed in the present work.

| Genus, species                  | Number of individuals | Locality               | Hydrographic basin | GPS data   | Diploid number | Karyotype formula | FN  | S   |
|---------------------------------|-----------------------|------------------------|--------------------|--|----------------|-------------------|-----|-----|
| <i>Pimelodella boschmai</i>     | 23♂, 27♀              | Araras -SP             | Mogi-Guaçu         | S <sub>0</sub> 22°22,994'<br>W <sub>0</sub> 47°25,825' | 46             | 38m + 8sm         | 92  | 0   |
| <i>Pimelodella meeki</i>        | 2♂, 2♀, 2?            | Pilar do Sul - SP      | Paranapanema       | S <sub>0</sub> 23°48,800'<br>W <sub>0</sub> 47°42,500' | 46             | 28m + 12sm + 6st  | 92  | 0   |
|                                 | 2♂, 12♀               | S. Miguel Arcanjo - SP | Paranapanema       | S <sub>0</sub> 23°54,743'<br>W <sub>0</sub> 47°57,675' | 46             | 28m + 12sm + 6st  | 92  | 0   |
| <i>Pimelodella lateristriga</i> | 7♂, 10♀               | Angra - RJ             | Paraíba do Sul     | S <sub>0</sub> 22°53,190'<br>W <sub>0</sub> 44°16,403' | 58             | 36m + 22sm        | 116 | 0   |
| <i>Pimelodella gracialis</i>    | 10♀                   | Mariápolis - SP        | Paraná             | S <sub>0</sub> 20°30,607'<br>W <sub>0</sub> 51°18,481' | 46             | 34m + 12sm        | 92  | 0   |
| <i>Pimelodella</i> sp.          | 1♂, 2♀                | Cardoso - SP           | Pardo              | S <sub>0</sub> 20°11,682'<br>W <sub>0</sub> 49°41,602' | 46             | 34m + 12sm        | 92  | 0-4 |

Legend: ? = individuals of unidentified sex, S = supernumerary chromosomes

somes, equivalent to the pair 21 in *P. boschmai*, 18 in *P. gracialis*, 20 in *P. lateristriga*, 20 in *P. meeki* and 21 in *Pimelodella* sp. These sites were positively fluorescent after CMA<sub>3</sub> staining, revealing a GC-

rich content. In *Pimelodella* sp. small additional fluorescent signals were observed at the pericentromeric region of supernumerary chromosomes, but undetected by silver nitrate staining (Fig. 2).

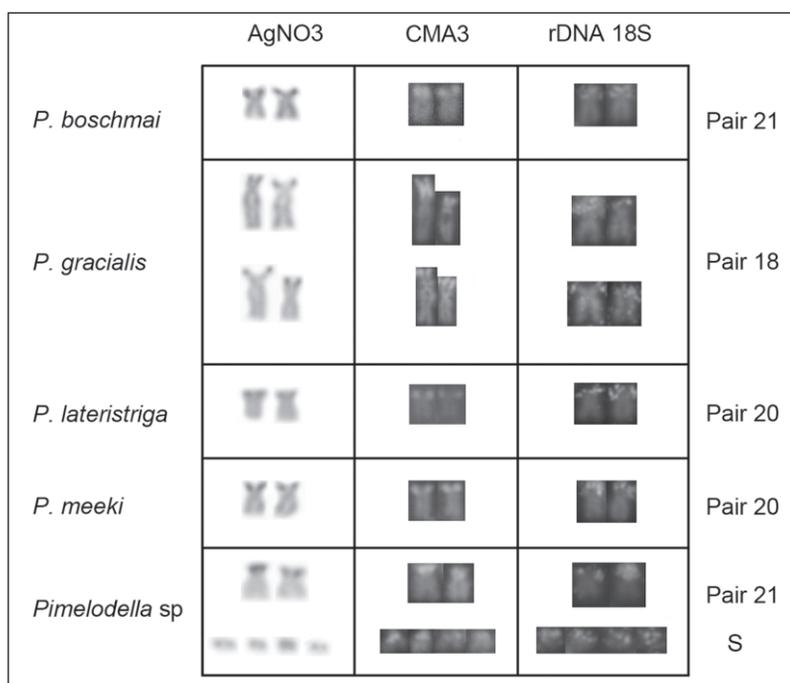


Fig. 2 — Nucleolar chromosomes of *Pimelodella* species after silver nitrate and CMA<sub>3</sub> staining and FISH with 18S rDNA probe. Presence of structural polymorphism in *P. gracialis* and additional CMA<sub>3</sub> and 18S rDNA sites at pericentromeric region of B chromosomes in *Pimelodella* sp. that were not evidenced by Ag-NOR.

FISH using 18S rDNA probes showed marks equivalent to those obtained by silver nitrate and CMA<sub>3</sub> staining, besides fluorescent signals at the pericentromeric region of the supernumerary chromosomes in *Pimelodella* sp. (Fig. 2).

The C-banding revealed a small amount of constitutive heterochromatin in the studied species. Reduced C-bands were preferentially distributed at pericentromeric regions of most chromosomes (Fig. 3a-e). In *P. boschmai*, a conspicuous C-band was observed at the terminal region on long arms of one homologous in the first chromosomal pair. This trait was restricted to males, but not to all of them, since only 13 individuals of the 23 males analysed presented this heterochromatic block, characterizing a male-related heterochromatin polymorphism in this population (Fig. 3a).

*P. gracialis* presented two chromosomal pairs bearing interstitial C-bands (Fig. 3c). Some individuals of this species lacked the interstitial heterochromatic block in one chromosome of the large NOR-bearing submetacentric pair causing a clear size difference between homologous (Fig. 3d). The NORs were heterochromatic in all analyzed species. On the other hand, the supernumerary chromosomes found in *Pimelodella* sp. proved to be euchromatic after C-banding (Fig. 3g).

*In situ* hybridization with 5S rDNA revealed fluorescent signals on short arms of a pair of submetacentric chromosomes in the populations of *P. boschmai* (pair 20) (Fig. 4a) and *P. meeki* (pair 19) (Fig. 4d). *P. lateristriga* presented a pair of 5S rDNA sites at the pericentromeric region on long arms of a metacentric pair (pair 14) (Fig. 4c). *P. gracialis* presented five sites located on submetacentric chromosomes; in two pairs (18 and 22) the marks were located on short arms and another signal was detected close to the centromere on long arms of a medium submetacentric chromosome (one homologous of the pair 19) (Fig. 4b). *Pimelodella* sp. presented three 5S rDNA sites, being two located on short arms of a submetacentric pair (22) and another at terminal position on long arms of a medium metacentric chromosome (one homologous of the pair 6) (Fig. 4e). The synteny of 5S and 45S rDNA sites were excluded after sequential silver nitrate staining of the FISH with 5S rDNA probes preparation (data not shown)

## DISCUSSION

The cytogenetic data available so far in the genus *Pimelodella* indicate three trends in rela-

tion to the diploid number: (1)  $2n = 46$  chromosomes, reported in most of analyzed species and populations (SWARÇA *et al.* 2000; present work); (2)  $2n = 52$  chromosomes, found in just two species, *Pimelodella* sp. (VASCONCELOS and MARTINS SANTOS 2000) and *P. aff. avanbandavae* (SWARÇA *et al.* 2003); and (3)  $2n = 58$  chromosomes, reported for *P. transitoria* and *P. kronei* (ALMEIDA-TOLEDO 1992) and *P. lateristriga* (present work).

Several authors refer to  $2n = 56 + 2$  chromosomes as the ancestor diploid number for Sil-

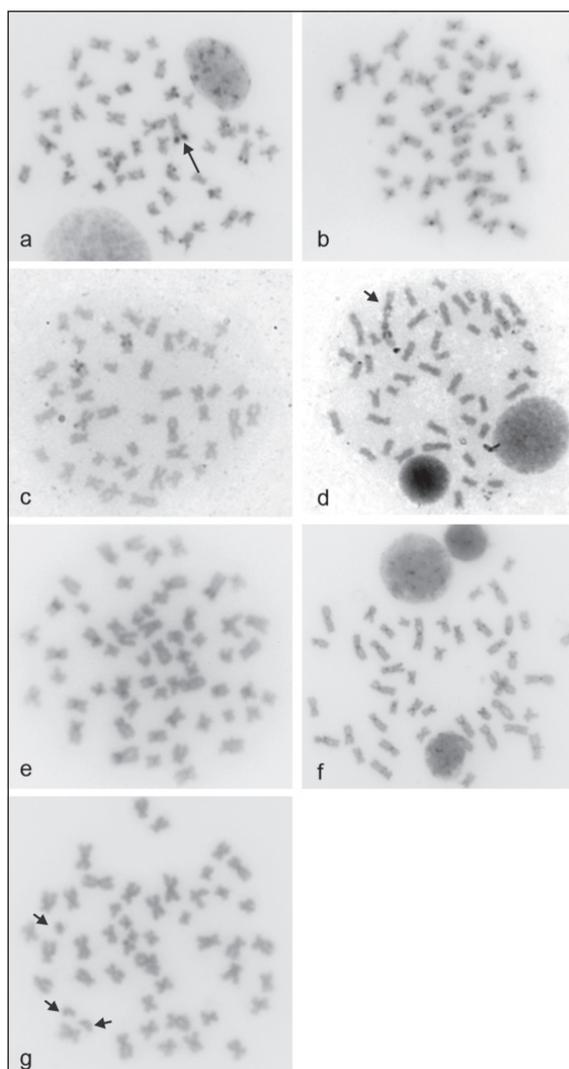


Fig. 3 — Somatic metaphases submitted to C-banding : (a) male of *P. boschmai*; the arrow indicates the polymorphic heterochromatin block; (b) female of *P. boschmai*; (c) *P. gracialis*; (d) polymorphism present in some individuals of *P. gracialis*, the arrowhead shows the presence of only a single heterochromatin block in one homologous of the 18<sup>th</sup> chromosomal pair; (e) *P. lateristriga*; (f) *P. meeki* and (g) *Pimelodella* sp., the arrowheads indicate the euchromatic B chromosomes.

uriformes (OLIVEIRA and GOSZTONYI 2000). Even though the diploid number  $2n=46$  is the most frequent one within *Pimelodella*, further genetic data are still required to reveal whether such diploid number is a plesiomorphic condition or a derived trait in this group, since higher diploid numbers, albeit rare, are also reported for *Pimelodella* representatives.

A subtle difference in the karyotype was also observed for *P. meeki* when compared to previously studied populations. Specimens from three populations in Tibagi River basin presented the same karyotypic formula  $30m + 12sm + 4st$  (VIDOTTO *et al.* 2004), whereas the populations presently analyzed presented  $28m + 12sm + 6st$ . This difference might be related to the occurrence of chromosomal inversions thereby differentiating the populations from Paraná River basin.

In the present work, peculiar chromosomal features were also detected in the populations bearing  $2n=46$  chromosomes, such as heterochromatin location, presence of supernumerary chromosomes and sex-related chromosomal systems.

Overall, the heptapterids present small amounts of heterochromatin mainly distributed over terminal and pericentromeric regions (VISOTO *et al.* 1999a; FENOCCHIO *et al.* 2000). C-bands located at interstitial position, although less frequent are typical of some genera like, for instance, *Imparfinis* (MARGARIDO and MOREIRA FILHO 2008). The occurrence of interstitial heterochromatic blocks in *Pimelodella* allows differentiating species and populations, such as *P. gracialis* that, albeit presenting the same chromosomal constitution of *Pimelodella* sp., can be distinguished by the presence of a conspicuous interstitial mark on pair 18.

The occurrence of a polymorphism involving the heterochromatin segments on the 18<sup>th</sup> chromosomal pair of *P. gracialis* was also detected. Such polymorphism, related to the absence of heterochromatin in one homologous, causes a size difference between the chromosomes in this pair. A similar situation was reported by VASCONCELOS and MARTINS-SANTOS (2000) in *Pimelodella* sp1, comprising the 12<sup>th</sup> chromosomal pair, where the deletion of a segment in one homologous was confirmed by G banding.

Heterochromatin polymorphism was also found in the first chromosomal pair of *P. boschmai*. This polymorphism, involving the presence of a conspicuous terminal C-band on long arms of one homologous in the largest metacentric pair was restricted to some males in the studied popu-

lation, always displaying a heterozygous situation. Once the sample number can be considered satisfactory, we suggest this population is currently under directional selective pressure where the homozygote form (i.e. two terminal heterochromatic blocks) has been negatively selected.

Studies focused on supernumerary chromosomes in fish are recent, while data about the occurrence of this chromosome type in plants and other animals has been known for a long time. One of the first reports about supernumerary or B-chromosomes in Neotropical fish was published by PAULS and BERTOLLO (1983) in *Prochilodus lineatus* (cited as *Prochilodus scrofa*). Little is known about the origin and the putative role of supernumerary chromosomes, since many factors can lead to differences in the frequency and morphology of these chromosomes.

The presence of supernumerary chromosomes in the species studied in the present work might be regarded as a homoplasmy in relation

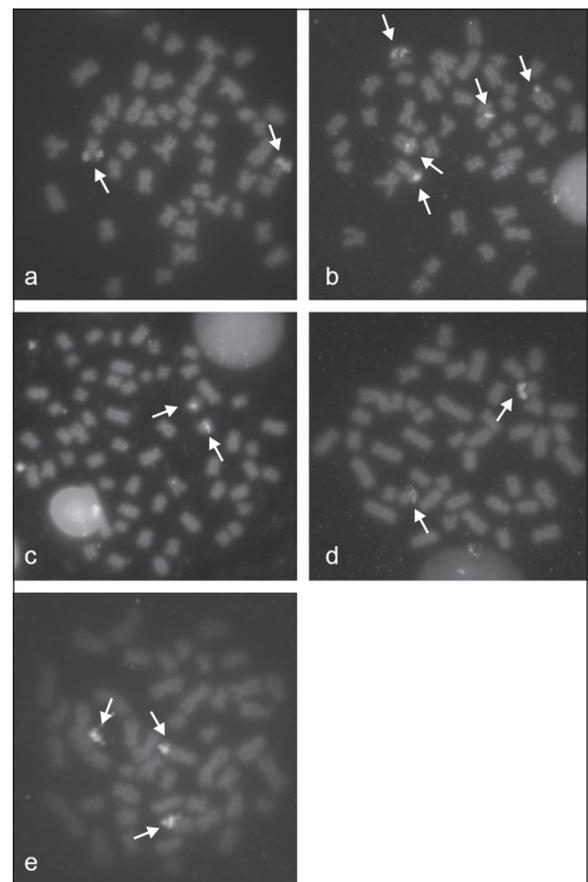


Fig. 4 — Somatic metaphases of (a) *P. boschmai*; (b) *P. gracialis*; (c) *P. lateristriga*; (d) *P. meeki* and (e) *Pimelodella* sp. after FISH with 5S rDNA probe. The arrows indicate the fluorescent sites.

to the family Pimelodidae and the new families Heptapteridae and Pseudopimelodidae. The presence of these chromosomes is commonly described in the genus *Rhamdia* (GARCIA *et al.* 2003; MORAES *et al.* 2009), and, just recently, they have been reported in other genera such as *Bergiaria* (DIAS and FORESTI 1993), *Iheringichthys* (VISSOTO *et al.* 1999b), *Pimelodella* (ALMEIDA-TOLEDO *et al.* 1992) and *Pimelodus* (BORIN and MARTINS-SANTOS 2004). There are no reports about supernumerary chromosomes in Pseudopimelodidae.

The supernumerary chromosomes found in *Pimelodella* sp. are microchromosomes that vary in number among cells of a same individual and proved to be euchromatic after C-banding. These features differ from those reported in *P. kronei* (ALMEIDA-TOLEDO *et al.* 1992), which presented just a single and entirely heterochromatic supernumerary element.

The supernumerary chromosomes of *Pimelodella* sp. also presented positive marks after CMA<sub>3</sub> staining and 18S rDNA FISH in regions previously undetected by silver nitrate staining. These results suggest a partial homology of these sites with the 18S rDNA probe and a lack of activity of these regions, what might correspond to the occurrence of pseudogenes. However, further studies are required to support this hypothesis. The presence of NORs on sex chromosomes (BORN and BERTOLLO 2000; ARTONI and BERTOLLO 2002) or on supernumerary chromosomes (DANTAS *et al.* 2007) is a rare event in fish, being this the first report of such occurrence in Siluriformes.

In the present work, the submetacentric NOR-bearing chromosomes of *P. gracialis* presented a larger size when compared to the nucleolar chromosomes of other analyzed populations. Such size difference is associated with the presence of a large heterochromatic block located on long arms of this chromosomal pair, which was also polymorphic as previously mentioned. In the polymorphic individuals lacking this heterochromatic block in one homologous, the chromosome missing such segment had the same shape and size of the NOR-bearing chromosomes commonly reported for the genus.

Therefore, the appearance of this large interstitial heterochromatic block in the NOR-bearing chromosome might putatively represent a recent event, not fixed in the population, what would explain the occurrence of heterozygous forms that determined the size polymorphism of such chromosomal pair. A more conclusive evolutionary explanation for the occurrence and

maintenance of this polymorphism within the studied population could be drawn by increasing the sample number as an attempt to find homozygous forms.

In general, the NOR-bearing chromosomes in *Pimelodella* are medium-sized submetacentric elements, where the size is usually quite similar among populations and the NORs present a heterochromatic nature. These findings were also confirmed by 18S rDNA FISH and CMA<sub>3</sub> staining. The relationship between NORs and GC-specific fluorochromes has been reported for several fish groups (SWARÇA *et al.* 1999; GARCIA *et al.* 2003). In most cases, the CMA<sub>3</sub> staining or mithramycin yields only fluorescent marks coincident to NORs, whereas some reports showed that heterochromatic regions can also be detected by such fluorochromes (GARCIA and MOREIRA-FILHO 2005). The populations analyzed in the present work presented GC-rich NORs, following a pattern already described for other *Pimelodella* species (SWARÇA *et al.* 2003; VIDOTTO *et al.* 2004).

Differentiated sex chromosomal systems are scarce in fish. Within Siluriformes, 10 cases of sex chromosomes have been reported so far and most of them were restricted to the family Loricariidae (SWARÇA *et al.* 2006). The first description of sex chromosomes in Heptapteridae was reported by DIAS and FORESTI (1993) in *Pimelodella* sp.; this species was characterized by the presence of a single sex chromosome system of XX/XY type, where the X chromosome was a medium-sized submetacentric and the Y was a small metacentric chromosome. In the present work, *P. boschmai* also presented a XX/XY sex chromosome system. However, the X was a small metacentric chromosome while the Y chromosome was a medium-sized metacentric element, thereby representing a rare situation in which the Y chromosome is larger than the X, as also detected in *Steindachneridion* sp. (SWARÇA *et al.* 2006).

Despite of the difference in the sex chromosomal pair between *Pimelodella* sp and *P. boschmai*, both species belong to Mogi-Guaçu River basin and they constitute the only cases of sex-related chromosomal differentiation within the genus. The resemblance of the karyotypic structure of these two species indicates their close relatedness. Possibly, there are some environmental and/or physical conditions in this basin that favor the chromosomal rearrangements to occur, leading to an isolation process by the differentiation of sex chromosomes. Similar evidence,

involving the identification of distinct sex chromosome systems within the same hydrographic basin was reported in the genus *Eigenmannia* from Upper Paraná basin (for a review see ALMEIDA-TOLEDO *et al.* 2001).

Studies involving the mapping of 5S ribosomal genes have shown variable results in relation to the number and localization of this marker, including the possible occurrence of synteny with 45S rDNA in different fish orders (GALETTI JR. and MARTINS 2004).

The majority of the Neotropical fishes analyzed so far present two 5S rDNA-bearing pairs, usually located at interstitial position, what has been commonly referred as a protective mechanism against deleterious chromosomal rearrangements (MARTINS and GALETTI JR. 1999; 2000). However, some species of the genus *Astyanax* escape this pattern, by presenting a higher number of sites (*A. intermedius*, *A. giton* and *A. scabripinnis* – KAVALCO *et al.* 2004).

In different groups, like Siluriformes (GARCIA and MOREIRA-FILHO 2008) and Characiformes (VICENTE *et al.* 2001), the localization of 5S rRNA genes has proved to be an efficient tool to characterize and differentiate populations. The few data available about the application of this cytogenetic marker in Siluriformes indicate that they present a remarkable variability mainly related to differences in the number of 5S rDNA-bearing sites (KAVALCO *et al.* 2004; GARCIA and MOREIRA-FILHO 2008). This feature is not observed in other fish orders, commonly characterized by an inter- and intra-generic conservatism (GALETTI JR. and MARTINS 2004).

The present work represents one of the first reports about 5S rDNA FISH in *Pimelodella*. We observed that most of analyzed species bears only a single site located at terminal position on short arms of submetacentric chromosomes. Moreover, when the diploid number is higher than  $2n = 46$ , these sites tend to present interstitial location. The increased number of 5S rDNA sites in *P. gracialis* might be a result of transposition followed by the increasing in the number of gene copies. Nonetheless, the lack of information about the evolutionary trends of this gene class in the genus hinders a reliable inference. Despite of these constraints, it is noteworthy that such chromosomal markers is remarkably variable within *Pimelodella*, what contrasts to the scenario reported in other genera of this family, like *Rhamdia*, where both the number and location of 5S rRNA gene are usually conserved (GARCIA personal information).

The present data show that the genus *Pimelodella* presents interesting features to be addressed regarding the chromosomal differentiation, with identification of structural and numerical polymorphisms in some species. Besides, few out of many described species have been cytogenetically analyzed inasmuch as more studies in this field might potentially increase their still underestimated chromosomal diversity, and thus contribute to a better understanding about the processes related to differentiation of species and populations in this fish group.

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