

Cytogenetical analyses in three fish species of the genus *Pimelodus* (Siluriformes: Pimelodidae) from rio São Francisco: considerations about the karyotypical evolution in the genus

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Karyotypes and other chromosomal markers were investigated in three species of the catfish genus *Pimelodus*, namely *P. fur*, *P. maculatus* and *Pimelodus sp.*, from municipality of Três Marias, Minas Gerais, Brazil, using differential staining techniques (C-banding, Silver nitrate and CMA₃ staining). The diploid chromosome number was $2n = 56$ in *P. maculatus* and *Pimelodus sp.*, while in *P. fur* $2n = 54$. The karyotype of *P. fur* consisted in $32M + 8SM + 6ST + 8A$ with fundamental number (NF) of 100, that of *P. maculatus* $32M + 12SM + 12A$ with NF = 112, and that of *Pimelodus sp.* had $32M + 12Sm + 6ST + 6A$ with NF = 106. The nucleolar organizer regions (NORs) in all three species were invariably detected in telomeres of longer arm of the 20th chromosome pair. These sites were also positive after CMA₃ and C-banding. No heteromorphic sex chromosomes were detected and C-banding pattern was species specific. Inferences about the karyotype differentiation in *Pimelodus* and putative chromosomal rearrangements are hypothesized.

Cariótipos e outros marcadores cromossômicos de três espécies do gênero *Pimelodus* denominadas, *P. fur*, *P. maculatus* e *Pimelodus sp.*, do município de Três Marias, Minas Gerais, Brasil, foram analisados utilizando técnicas de coloração diferenciais (Bandamento C, coloração por nitrato de prata e CMA₃). O número diplóide observado foi $2n = 56$ em *P. maculatus* e *Pimelodus sp.*, enquanto em *P. fur* $2n = 54$. O cariótipo de *P. fur* consistiu em $32M + 8SM + 6ST + 8A$ com número fundamental (NF) igual a 100, o de *P. maculatus* $32M + 12SM + 12A$ com NF = 112, e o de *Pimelodus sp.* possuía $32M + 12Sm + 6ST + 6A$ com NF = 106. As regiões organizadoras nucleolares (RONs) foram detectadas invariavelmente, para as três espécies, na região telomérica do braço longo do 20^o par cromossômico, estas se mostraram positivamente coradas pela CMA₃ e heterocromáticas. Nenhuma diferença cromossômica entre os sexos foi detectada e o padrão de bandamento C foi espécie-específico. Inferências sobre a evolução cariotípica dentro do gênero *Pimelodus* e os possíveis rearranjos cromossômicos envolvidos neste processo são discutidos.

Key words: Fish cytogenetics, diploid number reduction, chromosomal rearrangements, chromosome banding, catfishes

Introduction

The rio São Francisco basin is the fourth most important Brazilian basin, comprising nearly 7,4% of national territory, which includes North, Northeastern and Center-Western regions (Paiva, 1982). According to Britski *et al.* (1988), 133 fish species were described in the São Francisco and 57 of them occur at Três Marias, including: endemic catfish species *Pimelodus fur* and *Pimelodus sp.*, and *P. maculatus*, also found in the rio Paraná basin. These three species are representatives of the family Pimelodidae and share several morphological similarities, being identified especially by their color patterns.

The family Pimelodidae is one of the most important speciose groups within Siluriformes that includes 31 recognized genera and 83 valid species (Lundberg & Littmanm, 2003). *Pimelodus*, with 24 species (Lundberg & Littmanm, 2003) is one of most cytogenetically studied genus of Pimelodidae. It is widespread throughout South American rivers, comprising endemic species, such as *P. ortomanni* (rio Iguaçu basin, Southern Brazil), as well as widely distributed ones, like *P. maculatus*.

The genus *Pimelodus* is known by the presence of the diploid number $2n = 56$ associated with great variation in karyotype composition (Swarça *et al.*, 2000). Only one case of structural polymorphism was detected in this genus (Dias & Foresti, 1993).

In the present work, cytogenetic analyses were carried out in three species of *Pimelodus* from rio São Francisco, in order to provide useful information for both chromosomal characterization of these species and identification of putative chromosomal rearrangements involved in the evolution of the group.

Material and Methods

Materials included the following three species of *Pimelodus*: *P. fur* (10 females + 6 males), *P. maculatus* (10 females + 3 males), and *Pimelodus sp.* (15 females + 10 males), from rio São Francisco, collected at the municipality of Três Marias, Minas Gerais, Brazil. Identification of some specimens as "*Pimelodus sp.*" followed the diagnostic characters presented by Britski *et al.* (1988). Voucher specimens are kept at the fish collection of Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil, under numbers: MZUSP 86908 - *P. fur*, MZUSP 86907 - *P. maculatus* and MZUSP 86909 - *Pimelodus sp.*

Mitotic chromosomes were obtained from kidney cells using the direct preparation technique modified from Bertollo *et al.* (1978). Chromosomal morphology was determined according to arm ratio as proposed by Levan *et al.* (1964). The fundamental number (FN) was determined considering metacentric (M), submetacentric (SM) and subtelocentric (ST) chromosomes as biarmed, and acrocentric (A) as uniarmed. Nucleolar organizer regions (NORs) were visualized by silver staining (Ag-NOR), according to Howell & Black (1980). The base-specific fluorochrome staining was performed according to Schmid (1980) using the GC-specific fluorochrome Chromomycin A₃ (CMA₃). The C-banding was performed as described by Sumner (1972).

Results

P. fur had $2n = 54$ and the karyotype composed of $32M + 8SM + 6ST + 8A$, and $FN = 100$ (Fig. 1a). *P. maculatus* $2n = 56$, the karyotype $32M + 12SM + 12ST$ and $FN = 112$ (Fig. 1b), *Pimelodus sp.* $2n = 56$, and the karyotype $32M + 12SM + 6ST + 6A$, and $FN = 106$ (Fig. 1c).

Single Ag-NORs located at terminal position of the long arms of 20th chromosome pair was observed in all three species (Fig. 1 boxes). An NOR-size heteromorphism between homologous in *P. fur* was also observed

C-banding pattern was peculiar for each species. The NOR sites were C-band positive. *P. fur* and *Pimelodus sp.* (Fig. 2a and 2c) had C-band positive blocks distributed over pericentromeric and terminal regions of most of chromosomes. In *Pimelodus sp.*, C-band positive segments were also observed in both terminal regions of a pair of M chromosomes. *P. maculatus* had the lowest amount of C-band positive segments, located in pericentromeric regions (Fig. 2b).

CMA₃ staining revealed positive fluorescent signals cor-

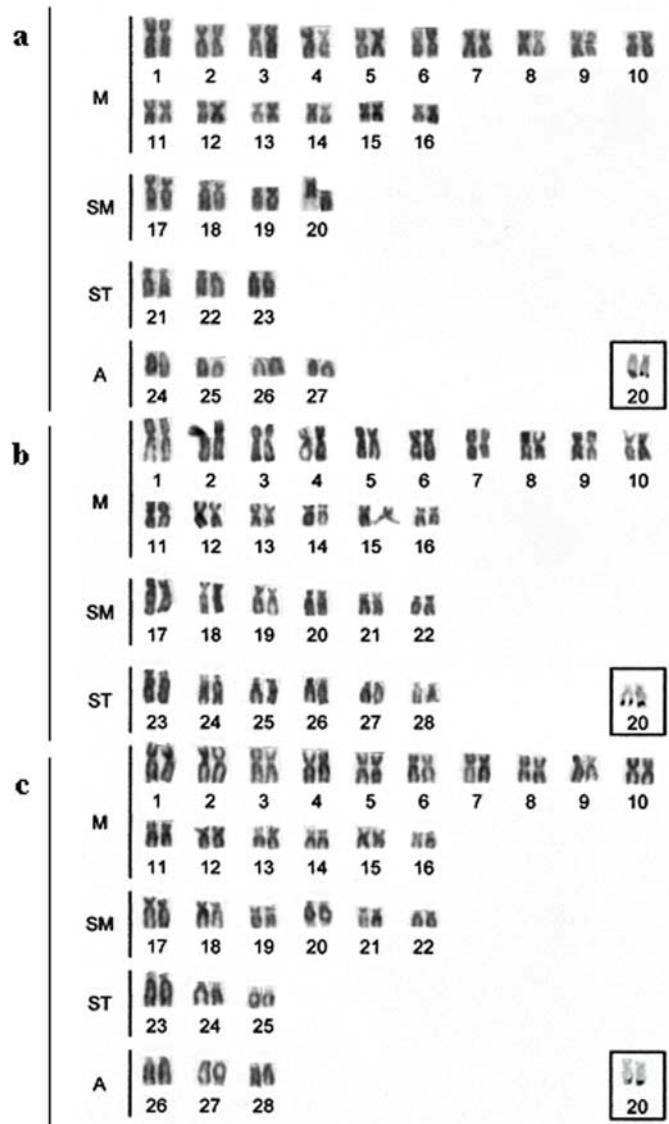


Fig. 1. Karyotypes of: a) *P. fur*, b) *P. maculatus*, and c) *Pimelodus sp.* arranged from Giemsa-stained. Ag-NOR-bearing pair is framed.

responding to Ag-positive signals in all three species. Moreover, other fluorescent signals corresponding with some other C-band positive segments were also observed in *Pimelodus sp.* (Figs. 2d, 2e, and 2f).

Discussion

The present work reports on the diploid number lower than $2n = 56$ among catfishes of the genus *Pimelodus*. The decrease in $2n$ in *P. fur* is likely due to the chromosomal rearrangement of fusion or translocation type. On the other hand, a diploid number higher than $2n = 56$ was reported for *P. blochii* where equals $2n = 58$ (Della-Rosa *et al.*, 1980).

Oliveira & Gosztonyi (2000) suggested that $2n = 56$ chro-

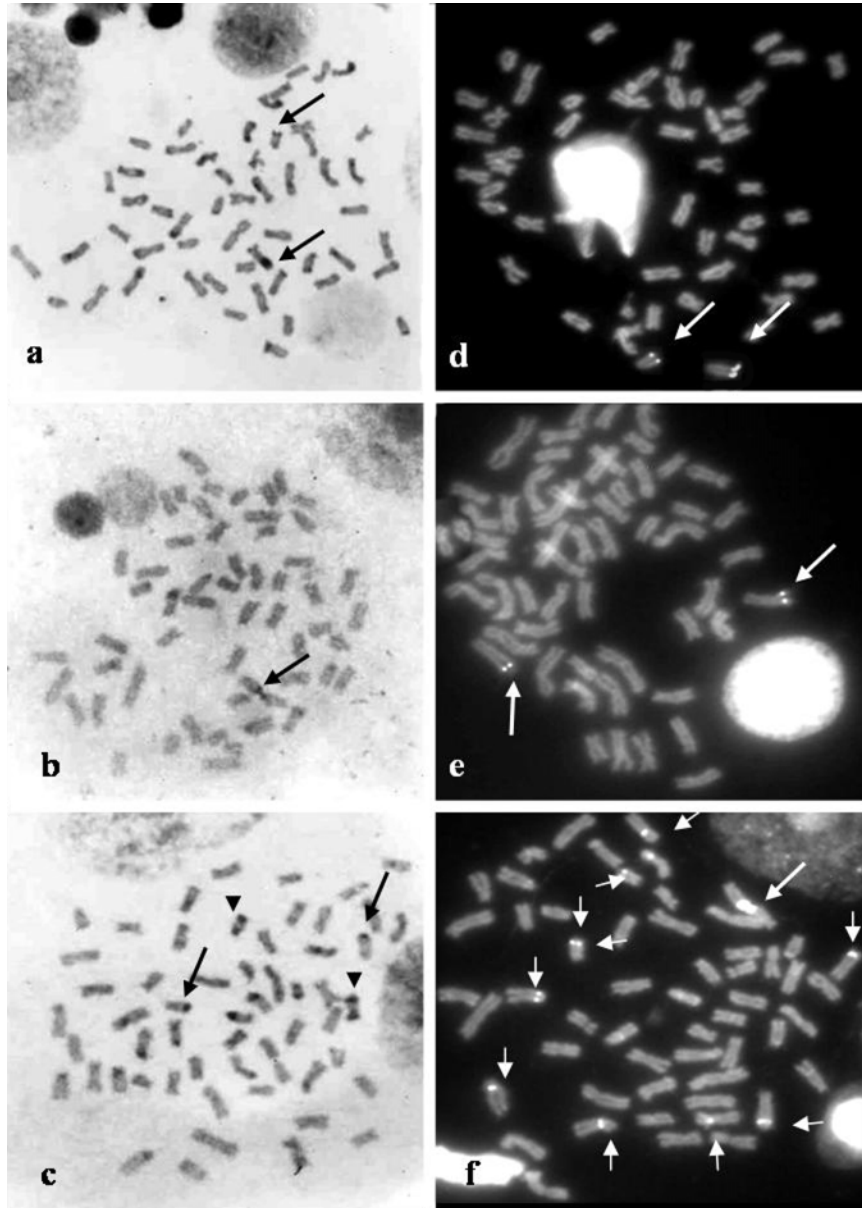


Fig. 2. Somatic chromosome metaphases of *P. fur* (a, d), *P. maculatus* (b, e) and *Pimelodus sp.* (c, f) submitted to: C banding (a, b, c) and staining with CMA₃ (d, e, f). The major arrows indicate the NOR-bearing pairs. NOR association (b, f) and NOR size heteromorphism (a) are exemplified. The arrows indicate positive chromosome regions corresponding with heterochromatin sites after treatment with fluorochrome and the arrowheads indicate a pair of metacentric chromosomes with both telomeres heterochromatic.

mosomes is the ancestral diploid number within Siluriformes. Therefore, the presence of this diploid number could be considered a plesiomorphic condition of the genus *Pimelodus*, also, while modifications (alterations) of $2n$ such as those detected in *P. fur* ($2n = 54$) and/or *P. blochii* ($2n = 58$) may represent rare and apomorphic characters.

Despite of the different diploid chromosome numbers, the species under study have similar if not identical karyotypes: all of them have the same number of M chromosomes (32) of similar size as well as NORs located at terminal position of longer arm of the 20th pair of chromosomes. This karyotype

uniformity might indicate their closely evolutionary relationship.

New observation is the absence of A chromosomes in the karyotype of *P. maculatus*. Specimens under study as yet, the lowest number of A chromosomes was one pair (*Pimelodus sp.* from Iguaçu river, Souza *et al.*, 2004a), whereas the highest was six pairs (*P. fur*, *P. maculatus* and *Pimelodus sp.*, Toledo & Ferrari, 1976). The most frequent pattern in the genus is the presence of three pairs of A chromosomes (Vissoto *et al.*, 1999; Swarça *et al.*, 2001; Borin & Martins-Santos, 2002) and the lack observed in *P. maculatus* could be a result of

non-Robertsonian rearrangements, such as pericentric inversions.

Pericentric inversions have been considered one of the major chromosomal rearrangements responsible for the karyotype variability in a number of fish groups, particularly in those with conservative uniform diploid number, such as *e.g.* Acestrorhynchinae and Cynopotaminae (Falcão & Bertollo, 1985), Serrasalminae (Cestari & Galletti Jr., 1992), Parodontidae (Jesus *et al.*, 1999; Jorge & Moreira-Filho, 2000), Trichomycteridae (Borin & Martins-Santos, 1999), Hypoptomatinae (Artoni & Bertollo, 2001), Pomacanthidae (Affonso *et al.*, 2002), Neoplecostominae (Kavalco *et al.*, 2005) among others.

Based on the available cytogenetic data for the family Pimelodidae, and Siluriformes as well, we hypothesize that pericentric inversions appear to be an important, if not the most common rearrangement karyotype differentiation in the family keeping the diploid number but diversifying the karyotype morphology.

Dias & Foresti (1993) have also analyzed *P. maculatus* and *Pimelodus sp.* from rio São Francisco. They reported the same karyotype composition for both species (40 M/SM + 16 ST/A), besides the occurrence of a structural polymorphism. Their results however do not agree with our data, differences between both reports can be attributed to the fact that chromosomes were assigned into particular categories on the basis of differently condensed chromosomes.

Ag-NOR analyses confirmed that the species under study retain the pattern observed in the genus, *i.e.*, a predominance of single Ag-NORs at terminal position of longer arm of ST chromosomes (Dias & Foresti, 1993; Vissoto *et al.*, 1999; Borin & Martins-Santos, 2002). On the other hand, the occurrence of Ag-NORs at chromosomal short arms appears to be relatively rare (Borin & Martins-Santos, 2002; Souza *et al.*, 2003).

The NOR sites of the three species were C-positive and thus heterochromatic, as also reported in another population of *P. maculatus* (Swarça *et al.*, 2001a). A size heteromorphism of these sites between homologues was frequently observed in *P. fur* after C-banding, CMA₃ and Ag-nitrate staining. Such size heteromorphism has been frequently reported in Pimelodidae (Gil *et al.*, 1998; Vissoto *et al.*, 1999; Swarça *et al.*, 1999, Souza *et al.*, 2004a, b). Because of their redundancy, the rDNA regions are likely more susceptible to unequal crossing-over or chromosomal rearrangements, leading to size differences between NOR-bearing homologous, particularly if these sites are associated to heterochromatic regions (Moreira-Filho *et al.*, 1984).

Correspondence between CMA₃ and Ag-NOR sites were observed in several pimelodid species, such as *Pirinampus pirinampu* (Swarça *et al.*, 1999), *Zungaro zungaro* (Swarça *et al.*, 2001b), *Pimelodus maculatus* (Swarça *et al.*, 2001a; Borin & Martins-Santos, 2002), *P. absconditus* and *P. ornatus* (Borin & Martins-Santos, 2002), and *P. argenteus* and *P. mysteriosus* (Souza *et al.*, 2003). In the present study, the same correspondence between CMA₃ and Ag-NOR positive sites was observed. Besides, other fluorescent signals were

detected in C-band positive chromatin of *Pimelodus sp.* documenting presence of heterochromatin of GC-type. This character shows that this species presents a distinctive heterochromatin classes than other two species. A similar situation was reported in *P. maculatus* from rio Paraguay (Souza *et al.*, 2003).

Species specific C-banding pattern permitted the determination of each species: *P. fur* and *Pimelodus sp.* had small C-band positive sites at the centromeric and terminal regions of some chromosomes, similarly to the pattern observed in this group (Vissoto *et al.*, 1999; Borin & Martins-Santos, 2002; Souza *et al.*, 2003) while *P. maculatus* had less C-band positive segments.

Nevertheless, a pair of M chromosomes bearing C-band positive segments at both telomeres were observed in *Pimelodus sp.* only. Similar C-banding pattern was reported in other species of the genus *Pimelodus* (*Pimelodus sp.* and *P. heraldoi* - Souza *et al.*, 2004a; *P. maculatus*, *P. ornatus* and *P. absconditus* - Borin & Martins-Santos, 2002) and some pimelodids and heptapterids (*Pimelodella sp.* - Vasconcelos & Martins-Santos, 2000; *Rhamdia sp.* - Garcia *et al.*, 2003; *Iheringichthys labrosus* - Carvalho *et al.*, 2004). This situation is frequently reported in these catfishes, indicating that the presence of this chromosome pair could be a shared condition and a cytotaxonomic marker for both families.

The present study shows, for the first time, two peculiar situations for representatives of the genus *Pimelodus*: the occurrence of a lower diploid number in *P. fur* and the absence of A chromosomes in *P. maculatus*. These findings might be related to intrinsic features of São Francisco River basin, which could favor the endemism of fish species in this basin. The present results contribute to the evolutionary and cytogenetic understanding of the phylogenetic relationships within this genus and the family Pimelodidae.

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