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Cytogenetic Analyses of *Pseudopimelodus mangurus* (Teleostei: Siluriformes: Pseudopimelodidae)

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Summary The cytogenetic analyses showed that *Pseudopimelodus mangurus* has a diploid number of $2n=54$ chromosomes (6M, 26SM, 12ST and 10A), single Ag-NORs on the short arm of a middle-sized ST pair, identified as pair 19, and a very small amount of C-band positive segments in two chromosome pairs. The Ag-NORs are C-band positive. The staining of the chromosomes of *P. mangurus* with CMA_3 reveled the occurrence of bright signals corresponding to the Ag-NORs segments, to the C-band positive segments and also to some C-band negative segments. The occurrence of a diploid number of $2n=54$ in all species of the family Pseudopimelodidae and its absence among representatives of Pimelodidae and Heptapteridae, two related families previously considered, reinforces the hypothesis that Pseudopimelodidae is a monophyletic group.

Key words Karyotype, chromosomes, Ag-NORs, C-band, Chromomycin A_3

The order Siluriformes (catfishes) has 2,405 species, divided into 34 families and 412 genera, worldwide distributed, except for the coldest areas in the Southern and Northern Hemispheres (Nelson 1994). Recent phylogenetic studies showed that the old family Pimelodidae comprised three monophyletic units: Pimelodidae, Heptapteridae and Pseudopimelodidae (Lundberg *et al.* 1991, de Pinna 1998, Britto 2003). Thus, Pseudopimelodidae with only 26 described species widely distributed in South America, can be considered the least known family among the naked Neotropical freshwater catfishes (Shibatta 2003). According to Shibatta (2003), Pseudopimelodidae is composed by the genera *Batrochoglanis* (4 species), *Cephalosilurus* (4 species), *Lophiosilurus* (1 species), *Microglanis* (12 species) and *Pseudopimelodus* (5 species).

Only the karyotypes of *Microglanis cottoides* (Vissotto *et al.* 1999a), *Lophiosilurus alexandri* (Marques *et al.* 2002) and *Pseudopimelodus bufonius* (Souza *et al.* 2003b) were so far described. The present study had as main objective characterizing the karyotype of *P. mangurus* and the data obtained were compared with those available from other species of Pseudopimelodidae, Pimelodidae and Heptapteridae.

Material and methods

Seven specimens of *Pseudopimelodus mangurus* (6 males, 1 female) from Mogi-Guaçu river (Pirassununga, São Paulo, Brazil) were karyotyped. Fishes were identified and deposited in the fish collection of the Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, São Paulo, Brazil.

Mitotic chromosome preparations were performed according to the technique described by Foresti *et al.* (1993). The silver-staining of nucleolar organizer regions (Ag-NORs) followed the technique proposed by Howell and Black (1980), C-banding was performed by the method of Sum-

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ner (1972), and chromosome staining with Chromomycin A_3 (CMA_3) was performed by the method of Schweiser (1976). The chromosome morphology was determined on the basis of arm ratios as proposed by Levan *et al.* (1964), and the chromosomes were classified according to the morphology as metacentrics (M), submetacentrics (SM), subtelocentrics (ST), and acrocentrics (A).

Results and discussion

P. mangurus showed a diploid number of $2n=54$ chromosomes (6M, 26SM, 12ST and 10A) (Fig. 1a). The presence of $2n=54$ chromosomes is a constant characteristic of the family Pseudopimelodidae, and the karyotypic formulae found in *P. mangurus* is similar of those from other species of this family since all karyotyped species have chromosomes of all types, except for *M. cottoides* that does not exhibit A chromosomes (Table 1).

The presence of $2n=54$ chromosomes in Pseudopimelodidae contrasts with the diploid numbers found in most other catfish families that exhibited a modal diploid number of $2n=56$ (Oliveira and Gosztonyi 2000) and mainly with the diploid number found among representatives of the families Heptapteridae and Pimelodidae, previously considered related to Pseudopimelodidae (Nelson 1994). Thus, for Heptapteridae, the occurrence of $2n=46$ chromosomes was described in *Pimelodella avanhandavae* (Vissotto *et al.* 1999a), *Pimelodella* aff. *meeki* (Dias and Giuliano-Caetano 2002), $2n=52$ in *Heptapterus longicauda* (Vissotto *et al.* 1999a), *Pimelodella* aff. *avanhandavae* (Swarça *et al.* 2003a), $2n=56$ in *Imparfinis* cf. piperatus (Vissotto *et al.* 2001), *Rhamdella microcephala* (Fonseca *et al.* 2003), and $2n=58$ in *Rhamdia quelen* (Hochberg and Erdtmann 1988, Fenocchio and Bertollo 1990), *Pimelodella kronei* and *Pimelodella transitoria* (Almeida-Toledo *et al.* 1992), *Imparfinis mirini* (Vissotto *et al.* 1997), *Cetopsorhamdia iheringi* (Vissotto *et al.* 1999a) and *Imparfinis piperatus* (Vissotto *et al.* 2001). Among Pimelodidae, $2n=50$ chromosomes were found in *Calophysus macropterus* (Ramirez-Gil *et al.* 1998), *Pirinampus pirinampu* (Swarça *et al.* 1999), and $2n=56$ in *Pseudoplatystoma fasciatum* and *P. tigrinum* (Fenocchio and Bertollo 1992), *Sorubim lima* (Fenocchio and Bertollo 1992), in *Bergiaria westermanni* (Dias and Foresti, 1993), *Pimelodus maculatus* (Dias and Foresti 1993), *P. argenteus* and *P. mysteriosus* (Souza et al. 2003a),

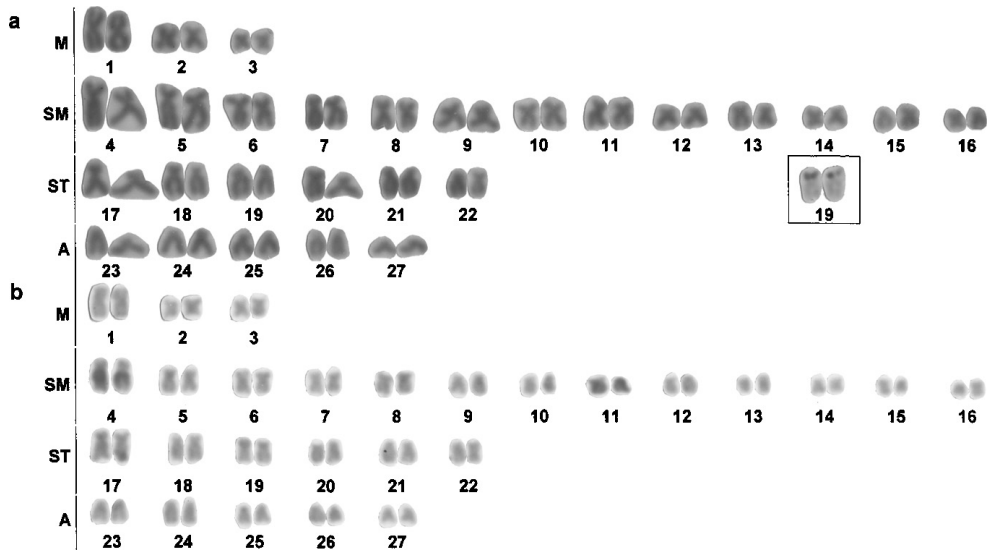

Fig. 1. Karyotypes of *Pseudopimelodus mangurus* with $2n=54$ chromosomes. (a) Giemsa stained and (b) C-banding. In the inset, silver stained chromosomes showing the terminal Ag-NOR on the short arm of the pair 19.

Table 1. Summary of the cytogenetic data available for the family Pseudopimelodidae. 2*n*=diploid number; M=metacentrics; SM=submetacentrics; ST=subtelocentrics; A=acrocentrics; NORs=number of chromosome pairs with nucleolus organizer regions.

Species	Locality	2*n*	Karyotype	NORs	References
Lophiosilurus alexandri	Três Marias reservoir, Minas Gerais, Brazil	54	54M, SM, ST, A	1	Marques *et al.* (2002)
Microglanis cottoides	Araquá and Capivara rivers, Botucatu, São Paulo, Brazil	54	22M+20SM+12ST	1	Vissotto *et al.* (1999)
Pseudopimelodus bufonius	Capim river, Ourém, Pará, Brazil	54	18M+22SM+6ST+8A	1	Souza *et al.* (2003b)
Pseudopimelodus mangurus	Mogi-Guacu river, Pirassununga, São Paulo, Brazil	54	6M+26SM+12ST+10A	1	Present study

Pseudoplatystoma corruscans (Martins-Santos *et al.* 1996), *Hemisorubim platyrhynchos* (Martins-Santos *et al.* 1996), Zungaro zungaro (Martins-Santos *et al.* 1996), *Iheringichthys labrosus* (Vissotto *et al.* 1999b), and *Steindachneridion* sp. (Swarça *et al.* 2003b). Thus, the presence of 2*n*=54 chromosomes may be an important characteristic to differentiate the species of Pseudopimelodidae from the species Heptapteridae and Pimelodidae.

P. mangurus exhibited single Ag-NORs on the short arm of a middle-sized ST pair, identified as pair 19 (Fig. 1a). The three remaining species of Pseudopimelodidae so far analyzed also have single Ag-NORs but *L. alexandri* has Ag-NORs on the short arm of a SM pair (Marques *et al.* 2002) and *M. cottoides* (Vissotto *et al.* 1999a) and *P. bufonius* (Souza *et al.* 2003b) have Ag-NORs on the long arm of an M pair. Single Ag-NORs were also identified in all species of Pimelodidae and all but one species of Heptapteridae analyzed until the present moment. This is also the most common condition in Siluriformes (Oliveira and Gosztonyi 2000) and even in Teleostei (Klinkhardt 1998).

C-banding showed the occurrence of a very small amount of C-band positive segments in the chromosomes of *P. mangurus* (Fig. 1b). Conspicuous C-band positive segments were only observed in the long arm of the largest SM pair (pair 4) and in the long arm of middle sized SM pair (pair 11). The Ag-NORs are C-band positive. The existence of a small amount of C-band positive segments in the chromosomes of *P. mangurus* and in other representative of the family Pseudopimelodidae, *Microglanis cottoides* (Vissotto *et al.* 1999a), suggests that this may be a characteristic of this catfish family. The occurrence of a very small amount of C-band positive segments resembles the data obtained for many other teleost species, including siluriforms (Gold *et al.* 1990).

The staining of the chromosomes of *P. mangurus* with CMA_3 (GC-specific) reveled the occurrence of bright signals in terminal position on the short arms of the third ST pair, corresponding to the Ag-NORs (Fig. 2). This correspondence between the two kinds of staining procedures has been related to a large number of species of fish (Amemiya and Gold 1986, Ràb *et al.* 1991, Artoni *et al*. 1999, Margarido and Galetti Jr. 2000). On the other hand, the analysis of the chromosomes of *P. mangurus* after the staining with CMA_3 showed that this fluorochrome stains more brightly the two segments identified positively by the C-band technique (Fig. 2), suggesting that these C-band positive segments may be GC-rich, as described for some species of fish (Artoni *et al.* 1999, Margarido and Galetti Jr. 2000). Additionally, some C-band negative segments appeared bright stained, as the terminal segments of the long arm of the smallest A chromosomes, suggesting that they also are GC-rich (Fig. 2).

Further analysis of additional species of Pseudopimelodidae with different staining techniques will provide important information for a better understanding of the chromosome evolution in the group and to confirm the conservative nature of the diploid number in this fish family.

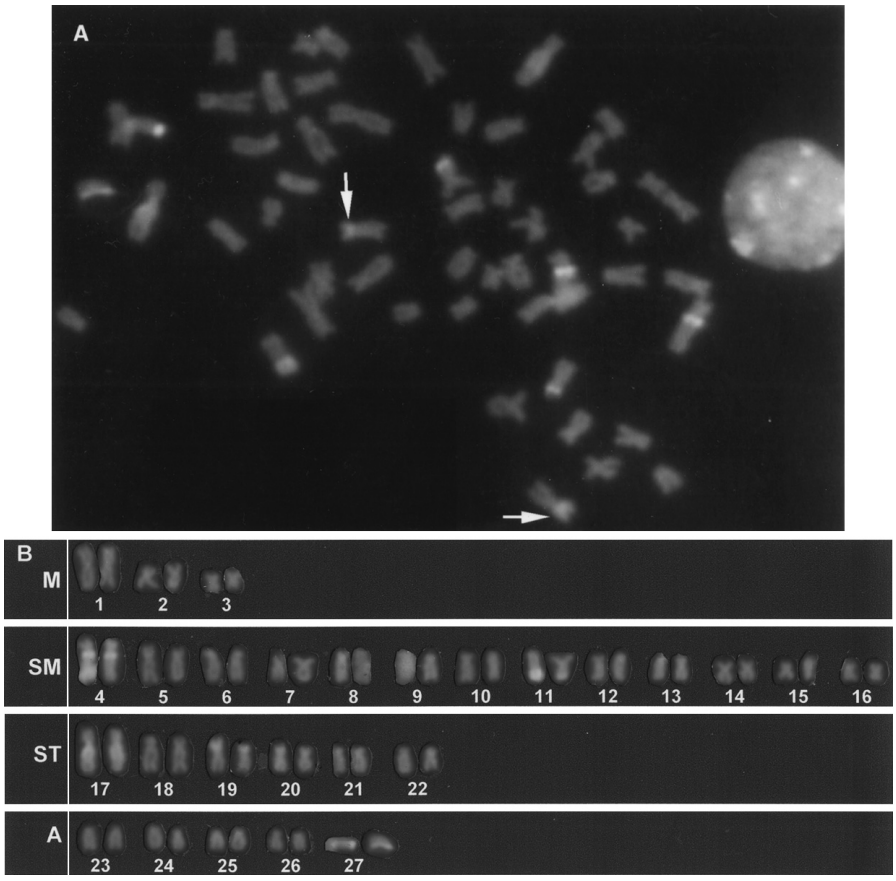

Fig. 2. Metaphase (A) and karyotype (B) of *Pseudopimelodus mangurus* stained with Chromomycin A_3. The arrows in (A) show the Ag-NOR-bearing chromosomes.

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