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Chromosomal analyses in *Megalonema platanum* (Siluriformes: Pimelodidae), an endangered species from South American rivers

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This study presents chromosomal data of *Megalonema platanum* from rio Tibagi, Paraná, Brazil and from rio Paraná, Argentina. The diploid number was equal 54 with karyotype composition of 24m+16sm+2st+12a in both populations. The AgNOR sites were detected in the terminal position of a submetacentric pair of the two analyzed populations, coinciding with secondary constrictions on the short arm of pair 15. CMA_3 and FISH with 18S rDNA probe displayed fluorescent signals that correspond to the AgNOR sites and secondary constriction. The presence of a small acrocentric supernumerary chromosome can be observed in *M. platanum* from rio Tibagi, with centromeric heterochromatin. Others heterochromatic blocks were evidenced in the terminal position of some chromosome and one metacentric large chromosome pair, probably the first pair, showed an interstitial heterochromatin. In the population of the rio Paraná were still observed heterochromatic blocks in both ends in some chromosomes. This work brings for the first time cytogenetic date of *M. platanum*, which is a very rare species in the rio Paraná basin and may be endangered.

Este estudo apresenta dados cromossômicos de *Megalonema platanum* do rio Tibagi, Paraná, Brasil e do rio Paraná, Argentina. O número diploide foi igual 54 com composição cariotípica de 24m+16sm+2st+12a em ambas populações. Os sítios AgNORs foram detectados na posição terminal de um par submetacêntrico das duas populações analisadas, coincidindo com constrição secundária no braço curto do par 15. CMA_3 e FISH com sonda de DNAr 18S exibiram sinais fluorescentes que correspondem aos sítios AgNORs e à constrição secundária. A presença de um pequeno cromossomo supranumerário acrocêntrico foi observado em *M. platanum* do rio Tibagi, com heterocromatina centromérica. Outros blocos heterocromáticos foram evidenciados na posição terminal de alguns cromossomos e um par cromossômico submetacêntrico grande, provavelmente o primeiro par, mostrou heterocromatina intersticial. Na população do rio Paraná foram observados ainda blocos heterocromáticos em ambas regiões terminais em alguns cromossomos. Este trabalho mostra pela primeira vez dados citogenéticos de *M. platanum*, que é uma espécie muito rara na bacia do rio Paraná e pode estar ameaçada de extinção.

Key words: Cytogenetics, C-banding, CMA_3, FISH, NORs, Pimelodidae.

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The genus *Megalonema* Eigenmann, 1910 is endemic to South America, for which there are few biological data available. Lundberg & Littmann (2003) defined six valid species of *Megalonema*: *M. argentina* (MacDonagh, 1938), *M. pauciradiatum* Eigenmann, 1919 and *M. platanum* (Günther, 1880) found in the rio Paraná basin; *M. platycephalum* Eigenmann, 1912 in the basins of the rios Amazonas, Essequibo and Orinoco; *M. psammium* Schultz, 1944 in the lago Maracaibo basin and *M. xanthum* Eigenmann, 1912 in the rio Magdalena basin. Recently Lundberg & Dadhul (2008) described more two species: *M. amaxanthum* n. sp. from the rio Amazonas basin and *M. orixanthum* n. sp. from the rio Orinoco basin. In *M. platanum* the body lacks clear and colored spots, with only the back of the head slightly darkened. The fins are clear, with the first ray of the pectoral ossified, and the snout is rounded and the eyes large (Lundberg & Littmann, 2003).

The present study presents the first karyotype description of *Megalonem platanum* using AgNOR conventional, fluorochrome staining, in additions to C-banding and fluorescence *in situ* hybridization (FISH) with 18S rDNA probe.

Twelve specimens of *M. platanum* were collected in distinct localities: 1) rio Tibagi, Paraná State, Brazil (23º21'27.22"S 51º00'34.36"W) (two males and two females); 2) río Paraná (28º57'30.72"S 57º25'52.32"W), from different localities in Argentina: Reconquista/Santa Fé (one male and two females), Corrientes/Corrientes (theree females), Yahapé/ Corrientes (one female) and Ita Ibaté/Corrientes (one female). The specimens of the rio Tibagi are deposited in Museu de Zoologia da Universidade Estadual de Londrina, voucher numbers MZUEL 1824 and of the rio Paraná are in Instituto de Ictiologia del Nordeste (Universidad Nacional del Nordeste, Argentina), voucher numbers INICNE 973, 1040, 1164, 1165, 1166, 1188, 1209, 1211. Mitotic chromosomes preparations were obtained according to Bertollo *et al*. (1978) and according to Foresti *et al*. (1993). Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a), according to Levan *et al*. (1964). NOR silver staining and C-banding were performed using the methods of Howell & Black (1980) and Sumner (1972), respectively. A rDNA 18S probe (1700 pb) obtained from the nuclear DNA of the fish *Oreochromis niloticus* (Linnaeus, 1758) was used for *in situ* hybridization and was carried out as described by Swarça *et al*. (2001). This probe was marked with biotin-14-dATP by nick translation (Gibco No. cat. 18247-015), according to the manufacturer's instructions. Chromomycin A_3 (CMA_3) staining followed Schweizer (1980).

The analysis of mitotic chromosomes of *M. platanum* showed a modal diploid number of 54 chromosomes in the two populations analyzed, with 24m+16sm+2st+12a (Fig. 1a). In some metaphases examined, we found a pair of submetacentric chromosomes (pair 15) with a secondary constriction at the short arm in the terminal position (Fig. 1a). In a review on the cytogenetic studies of 27 species of the family Pimelodidae by Swarça *et al*. (2007), 23 had a diploid number of 56 chromosomes, three had 2n = 50 chromosomes: *Pinirampus pirinampu* (Spix & Agassiz, 1829) (Swarça *et al*., 1999; Vasconcelos & Martins-Santos, 2000), *Luciopimelodus pati* (Valenciennes, 1835) (Sánchez, 2006), and *Calophysus macropterus* (Liechtenstein, 1819) (Ramirez-Gil *et al*., 1998), and one had 2n = 54 chromosomes: *Pimelodus fur* (Lütken, 1874) (Garcia & Moreira-Filho, 2005).

Three individuals of *M. platanum* from rio Tibagi showed the presence of an additional small acrocentric chromosome (Fig. 1a), indicating the occurrence of a supernumerary chromosomes. This chromosome was found to be present at a high intrapopulation frequency (75%) and a low intra-individual frequency (15.6%), where in a total of 96 metaphases examined in the population, only 15 had this small supernumerary chromosome. The absence of these chromosomes in the samples of lower rio Paraná basin suggested that these populations are isolated which should be confirmed through the analyses of additional specimens.

In Neotropical fishes, 61 species have supernumerary chromosomes or Bs, distributed in 16 families, 33 genera and seven different orders (Carvalho *et al*., 2008). These chromosomes in the family Pimelodidae were described in *Bergiaria westermanni* (Lütken, 1874) by Dias & Foresti (1993), *Iheringichthys labrosus* (Lütken, 1874) by Vissotto *et al*. (1999), Carvalho *et al*. (2004), and Carvalho & Dias (2005), *Pimelodus ortmanni* Haseman, 1911 and *Pimelodus* sp. NUP 690, NUP 1664, NUP 1786, NUP 1826 (NUP refers to the ichthyological collection of NUPELIA - Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá) by Borin & Martins-Santos (2004). The findings of the present study on *M. platanum* from rio Tibagi increases the number of species bearing supernumerary chromosomes to five for the family Pimelodidae, representing 8.2% of species carrying such chromosomes.

The C-banding technique allowed the detection in *M. platanum* of heterochromatic blocks in the terminal position of some chromosome pairs and a pair of large submetacentric chromosomes with interstitial heterochromatin in all populations (Fig. 1b, c). The individuals from rio Paraná also present some chromosomes with bands in both telomeric regions (Fig. 1c). C-banding for most of the family Pimelodidae is similar to that seen in *M. platanum* with some heterochromatic regions in the terminal position, as for example, in species of the genus *Pimelodus* (Souza *et al*., 2004; Garcia & Moreira-Filho, 2005; Treco *et al*., 2008), *Steindachneridion* Eigenmann & Eigenmann, 1919 (Swarça *et al*., 2005) and others. In several species and/or populations of the genus *Pimelodus,* chromosomal marker pairs, with interstitial heterochromatin, could also be seen (Borin & Martins-Santos, 2004; Treco *et al*., 2008).

Supernumerary chromosomes are generally heterochromatic; however, in this work, the acrocentric B-chromosome of *M. platanum* showed heterochromatin only in the centromeric

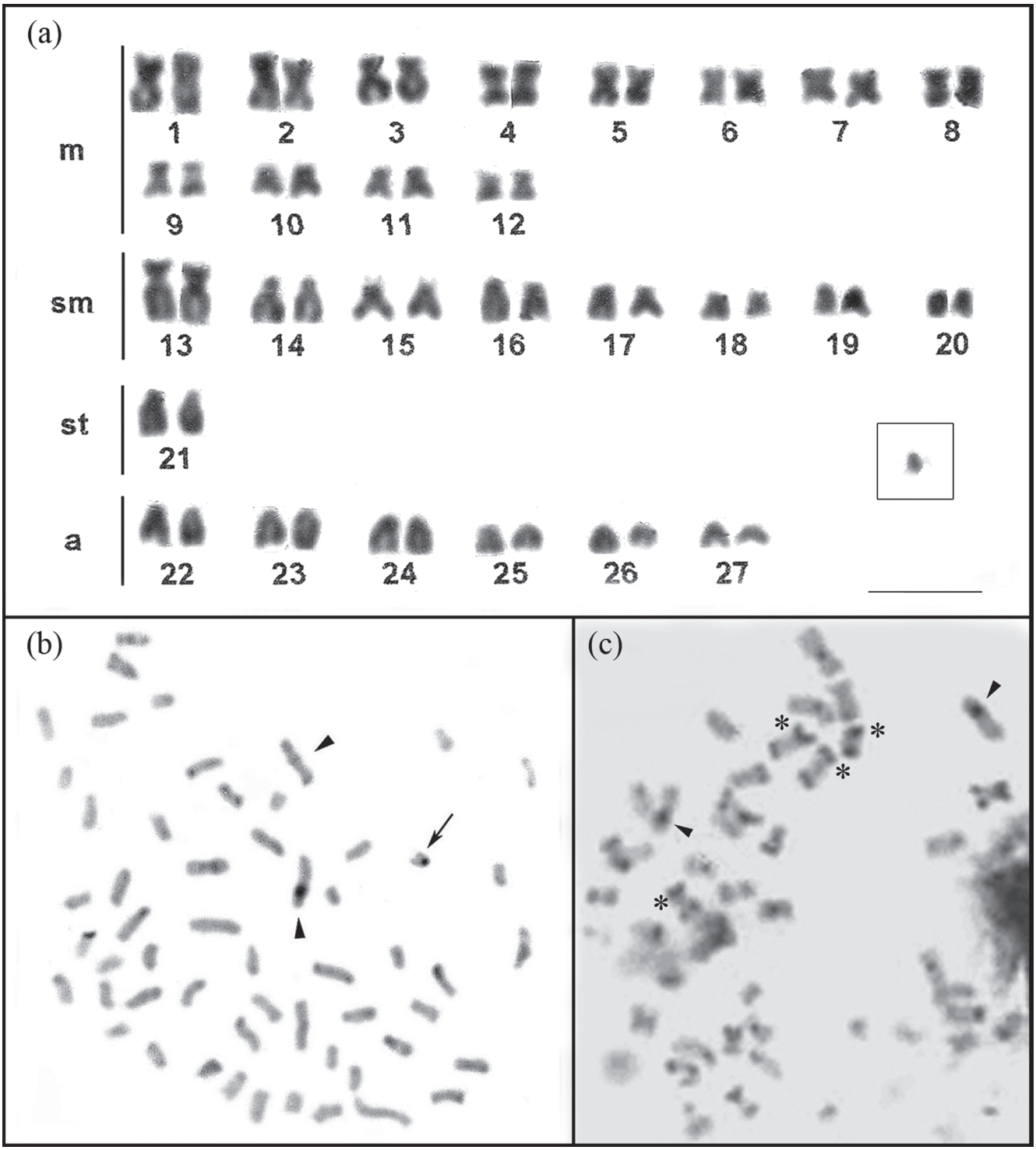

Fig. 1. Karyotype (**a**) and somatic metaphases of *Megalonema platinum* with C-banding (**b**) rio Tibagi and (**c**) rio Paraná. In the box the supernumerary chromosome. The arrowheads in (**b**) and (**c**) indicate the chromosome pair with interstitial heterochromatin; the arrow in (**b**) indicate the supernumerary chromosome partially heterochromatic and the asterisks in (**c**) indicate the chromosomes with heterochromatin blocks in both terminal regions. Scale bar = 10µm

region, thus partially heterochromatic (Fig. 1b). Some species of the genus *Rhamdia* Bleeker, 1858, were shown to have partially heterochromatic B chromosomes (Abucarma & Martins-Santos, 2001; Stivari & Martins-Santos, 2004).

The AgNORs sites were detected in the terminal position of a submetacentric pair of the two analyzed populations, coinciding with secondary constrictions on the short arm of pair 15 (Fig. 2a). In some metaphases, only one silver-stained

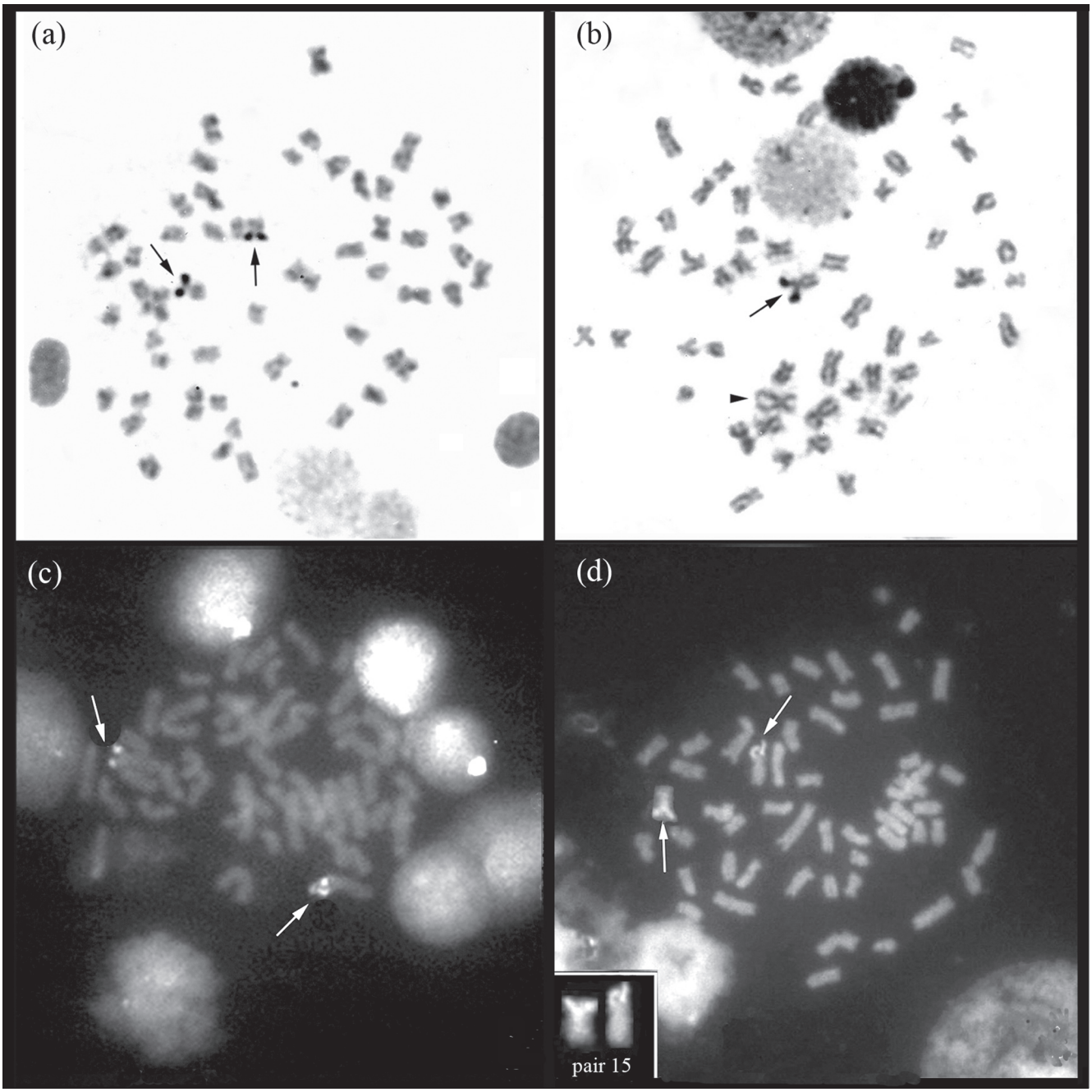

Fig. 2. Somatic metaphases of *Megalonema platanum* submitted to treatment with $AgNO_3$ (**a** and **b**); FISH with 18S rDNA probe (**c**) and CMA_3 (**d**). Arrows indicate the NOR bearing chromosomes. The arrowheads indicate the secondary constriction. Detail in (**d**) shows the chromosome pair CMA_3 positive.

chromosome was observed, indicating an inactive NOR in one of the homologous chromosomes (Fig. 2b).

In situ hybridization with 18S rDNA probe in *M. platanum* confirmed the NOR location in the secondary constriction of a pair 15 (Fig. 2c) as also observed in some Pimelodidae species by different authors: Souza *et al*. (2004), Carvalho & Dias (2007), Swarça *et al*. (2008), Garcia & Moreira Filho (2008).

CMA_3 staining on chromosomes of *M. platanum* displayed fluorescent signals on the short arm of a pair of sm chromosomes (Fig. 2d), probably coinciding with the AgNOR, showing that this region of secondary constriction is rich in GC base pairs since the fluorochrome CMA_3 preferably binds to chromatin segments rich in these base pairs. In these preparations, it was not possible to observe the supernumerary chromosome. In several species of fish, a positive correlation has been reported between of CMA_3 sites and AgNORs (Garcia & Moreira-Filho, 2005; Treco *et al*., 2008).

The data show that in terms of diploid number, *M. platanum* can be regarded as ancestral in relation to other species of Pimelodidae, and suggest that chromosomal

rearrangements such as Robertsonian translocation or chromosomal fusion/fission are involved in the evolution of this group of fish. Thus, *M. platanum* must represent a primitive group within the family, with 2n = 54 that is a feature rare and shared with Pseudopimelodidae that possibly is the sister group of Pimelodidae (Sullivan *et al*., 2006). Therefore 2n = 56 and 2n = 50, observed in other Pimelodidae species, could be derived karyotype characteristics. As for the microstructure, that is, number and location of the nucleolus organizer regions and the pattern of heterochromatin distribution, *M. platanum* has a more conservative character, even though individuals from rio Paraná present some peculiarities (*i.e*., heterochromatin in both telomeric regions).

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