Cytogenetics of *Imparfinis schubarti* (Siluriformes: Heptapteridae) from the Piumhi drainage, a diverted river in Minas Gerais State, Brazil

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ABSTRACT. Specimens of *Imparfinis schubarti* (Gomes, 1956) collected in the Piumhi river drainage, state of Minas Gerais, Brazil, were studied cytogenetically. The river was diverted from the Rio Grande basin into the São Francisco basin in the early 1960s. All individuals presented 2n = 58 chromosomes, including 18 metacentric, 34 submetacentric and six subtelocentric chromosomes. A secondary constriction was observed in the interstitial region of the long arm of the largest chromosome pair, coinciding with the NOR. A single conspicuous heterochromatic block located in the largest pair of metacentric chromosomes was observed, adjacent to the secondary constriction. A detectable 18S rDNA probe hybridization region occurs in only one chromosome pair and is syntenic with the marking obtained with 5S rDNA probe. These results fit the cytogenetic pattern previously described for the genus *Imparfinis* Eigenmann & Norris, 1900.

KEYWORDS. Transposed river; subclade nemuroglanis; FISH; São Francisco River Basin.
The mitotic metaphases were obtained according to Bertollo et al. (1978) and Foresti et al. (1993). Chromosome morphology was determined according to the arm size relation proposed by Levane et al. (1964). The fundamental number (FN) was established through the sum of the number of chromosome arms, counting two arms for metacentric (m), submetacentric (sm), and subtelocentric (st) chromosomes, and one arm for acrocentric (a) chromosomes. The constitutive heterochromatin was identified using the barium hydroxide method (Sumner 1972), and the nucleolar organizing regions were detected through silver nitrate staining (Howell & Black 1980). Each preparation was stained in conventional Giemsa staining, and subsequently submitted to C-banding.

The location of the 18S and 5S rDNA sites in the chromosomes was performed using the Fluorescence in situ Hibrization (FISH) technique (Pinkel et al. 1986), with 77% stringency and probes obtained from Prochilodus argenteus Spix & Agassiz, 1829 (Hatanka & Galetti Jr 2004) and Leporinus elongatus Valenciennes, 1850 (Martins & Galetti Jr 1999), respectively. The probes were marked with 14-dATP-biotin by nick translation according to the manufacturer’s instructions (Bionick Labelling System – Invitrogen). The chromosomes were counterstained with DAPI (0.2 mg/ml) and analyzed in an Olympus BX50 epifluorescence microscope. The software Image-Pro Plus (Media Cybernetics) was used for image capture.

RESULTS

The samples presented 2n = 58 chromosomes with 18m + 34sm + 6st (Fig. 2) and a fundamental number of 116. No gender-related chromosomal differences were observed. A conspicuous secondary constriction coinciding with the Ag-NOR was observed in the interstitial region of the long arm of the first submetacentric chromosome pair. Differences in the sites obtained through silver nitrate impregnation between the chromosomes of pair 1 were often observed (Fig. 3). The chromosomes have low quantities of constitutive heterochromatin, except for the first pair of submetacentric chromosomes, which has a large heterochromatic block adjacent to the secondary constriction (Fig. 3).

Fluorescence in situ hybridization with 18S rDNA probes produced markings that coincided with the AG-NORs located in the secondary constriction of the first pair of sub metacentric chromosomes. These markings were heteromorphic between homologous chromosomes (Fig. 4). The 5S rDNA FISH markings were synthenic with those for 18S rDNA (Fig. 5).
DISCUSSION

The 2n = 58 karyotype identified in *Imparfinis schubarti* is the most common diploid number among species of *Imparfinis* (Tab. I), as well as within the entire order Siluriformes (OLIVEIRA et al. 1988). Species of *Imparfinis*, as most Siluriformes, are also characterized by predominance of metacentric and submetacentric chromosomes and high fundamental number (FN) values. These features correspond to plesiomorphic conditions that are widely distributed throughout the order (OLIVEIRA & GOSZTONYI 2000). The FN = 116, found in *Imparfinis schubarti*, is the most common in the genus, being present also in *I. mirini* (Vissotto et al. 1997) and *Imparfinis sp. aff. I. schubarti* (STOLF et al. 2004).

Simple nucleolar organizing regions located in the largest chromosome pair coinciding with interstitial secondary constrictions and 2n = 58 are characteristics present in *I. schubarti*, *I. mirini*, *I. piperatus*-cytotypes A and B, and *Imparfinis sp. aff. I. schubarti* (Tab. I). If 2n = 58 is a plesiomorphic condition as suggested by OLIVEIRA et al. (1988) and FENOCCHIO et al. (2003), a reduction in the number of chromosomes may be a synapomorphy grouping *Imparfinis* sp. cf. *I. piperatus* from the Juquiá River (Vissotto et al. 2001, Fenocchio et al. 2003), *I. borodini* (Margarido & Moreira-Filho 2008) and *I. hollandi* (Vissotto et al. 1999). This hypothesis is corroborated by the repositioning of the nucleolar organizing region in these species from chromosome pair 1 to other pairs in these species. Additionally, the migration of the...

Figures 2-3. Karyotypes of *Imparfinis schubarti*: (2) Giemsa stain and (3) C-band. In the box, interstitial Ag-NOR in the largest chromosome pair.

Figures 4-5. *Imparfinis schubarti* metaphases. Chromosomal distribution (4) of the 18S rDNA and (5) of the 5S rDNA sites.
nucleolar organizing region from an interstitial to a terminal position may represent a synapomorphy further grouping *Imparfinis* and *I. borodini*. In superior eukaryotes, the 5S and 18S genes are frequently disposed in separate chromosome pairs (LONG & DAVID 1980, LUCCHINI et al. 1993, DROUIN & MONIZ DE SÁ 1995). This is the most frequent condition in fishes (MARTINS & GALETTI JR 2001) and represents the ancestral state of the chromosomal organization (MARTÍNEZ et al. 1996). The synthetic location of the 5S and 18S rDNA observed in *I. schubarti* is therefore an apomorphic condition, and further investigation on the distribution of this trait among species of *Imparfinis* may provide useful phylogenetic data.

The natural distribution of *I. schubarti* comprises the upper Paraná River Basin (BOCKMANN & GUZZELLI 2003, BOCKMANN 2007). However, the transposition of the River Piumhi in the early 1960s introduced several species from the upper Paraná into the São Francisco river basin (Fig. 1) (MOREIRA-FILHO & BUCKUP 2005). The presence of *I. schubarti* in the region of the transposition channel of the Piumhi River, now belonging to the São Francisco River Basin, emphasizes the importance of cytoge-
nomic and taxonomical studies involving native species of the São Francisco basin, such as Imparfinis minutes (Lütken, 1874). The present study represents a starting point in the evaluation of possible hybridization of such species with I. schubarti.

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