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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 synthenic 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.

Lundberg *et al.* (1991a, b) divided Pimelodidae into presumably monophyletic subfamilies, Pseudopimelodinae and Rhamdiinae, suggesting that the family is not monophyletic in its traditional definition. The hypothesis of Pimelodidae polyphyly was corroborated by Pinna (1998) based on phylogenetic studies of the order Siluriformes. Pinna (1998) also demonstrated the priority of the name Heptapterinae over Rhamdiinae. Bockmann & Guazzelli (2003) elevated Heptapterinae to the category of family, including 190 species and 24 genera. Subclades of Heptateridae have been recognized in the literature based on morphological data (Ferraris 1998, Lundberg *et al*. 1991a, Bockmann 1994), and *Imparfinis* has been included in the so-called *Nemuroglanis* subclade, along with thirteen other genera: *Acentronichthys* Eigenmann & Eigenmann, 1889, *Cetopsorhamdia* Eigenmann & Fisher in Eigenmann, 1916, *Chasmocranus* Eigenmann, 1912, *Heptapterus* Bleeker, 1858, *Horiomyson* Stewart, 1986, *Imperales* Schultz, 1944, *Medemichthys* Dahl, 1961, *Nannorhamdia* Regan, 1913, *Nemuroglanis* Eigenmann & Eigenmann, 1889, *Pariolius* Cope, 1872, *Phenacorhamdia* Dahl, 1961, *Phreatobius* Goeldi, 1905, and *Rhamdiopsis* Haseman, 1911.

Imparfinis, with 18 valid species (Bockmann & Guazzelli 2003), is one of the least phylogenetically resolved groups of Heptapteridae (Bockmann 1994). Species of *Imparfinis* inhabit the headwaters of rivers from Central America and the tropical areas of South America. They are of small in size and have benthic and nocturnal habits (Castro & Cassati 1997).

Up to this moment, six species of *Imparfinis* have been studied cytogenetically: *Imparfinis mirini* Haseman, 1911, with 2n = 58; *I. borodini* Mees & Cala, 1989, with 2n = 52; *I. piperatus* Eigenmann & Norris, 1900, with 2n = 58; *Imparfinis* sp. cf. *I. piperatus* with 2n = 56; *Imparfinis* sp. aff. *I. schubarti* with 2n = 58; and *I. hollandi* Haseman, 1911 with 2n = 42 (Tab. I). In these species there is a predominance of metacentric and submetacentric chromosomes and a simple interstitial NOR.

In this paper we describe the karyotype of a population of *I. schubarti* from the Piumhi River basin.

The River Piumhi, a tributary of the River Rio Grande in state of Minas Gerais, Brazil, was diverted in the 1960s from the Rio Paraná basin into the São Francisco basin. During construction of the Furnas hydroelectric dam in the Rio Grande, a secondary dike was built in the lower course of the River Piumhi in order to raise the water volume of the Furnas Dam and to avoid the flooding of the town of Capitólio. This secondary damming required the deviation of the Piumhi from the Rio Grande drainage into the São Francisco basin (Moreira-Filho & Buckup 2005) (Fig. 1). This study is part of a comprehensive investigation on the mixture of Paranean and San Franciscan fish faunas that resulted from the diversion of the River Piumhi.

MATERIAL AND METHODS

Ten specimens (four males and six females) collected in the Ribeirão Minhocas (20°31'55.2"S, 46°02'42.1"W), a small tributary of the River Piumhi (Fig. 1), were cytogenetically studied in this work. Voucher specimens are deposited in the Ichthyological Collection of the National Museum under number MNRJ 29336 (2 specimens) and MNRJ 32759 (8 specimens).

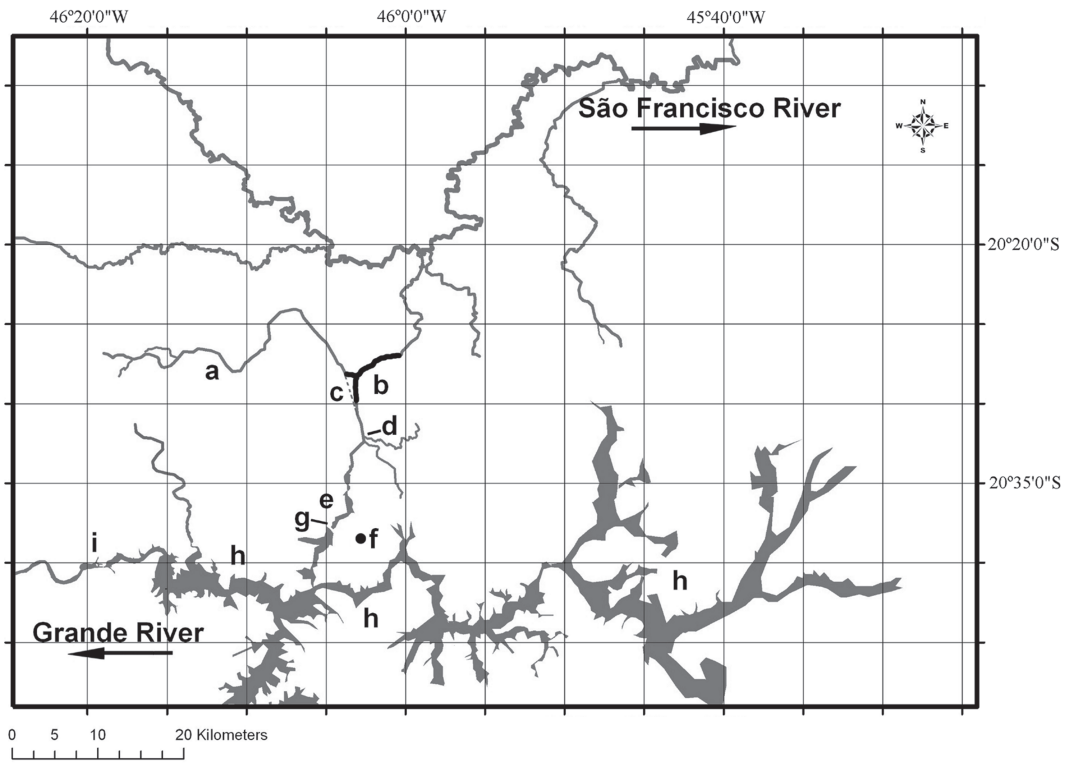


Figure 1. Transposition region of the Piumhi river: a) Piumhi River, b) transposition channel (thick black lines), c) old channel of the Piumhi River (dashed line), before the transposition, d) collection site, e) portion of Piumhi River dammed after construction of the dike near Capitólio, f) Town of Capitólio, g) Capitólio dike, h) Furnas Lake, and i) Grande river downstream from the Furnas hydroelectric dam.

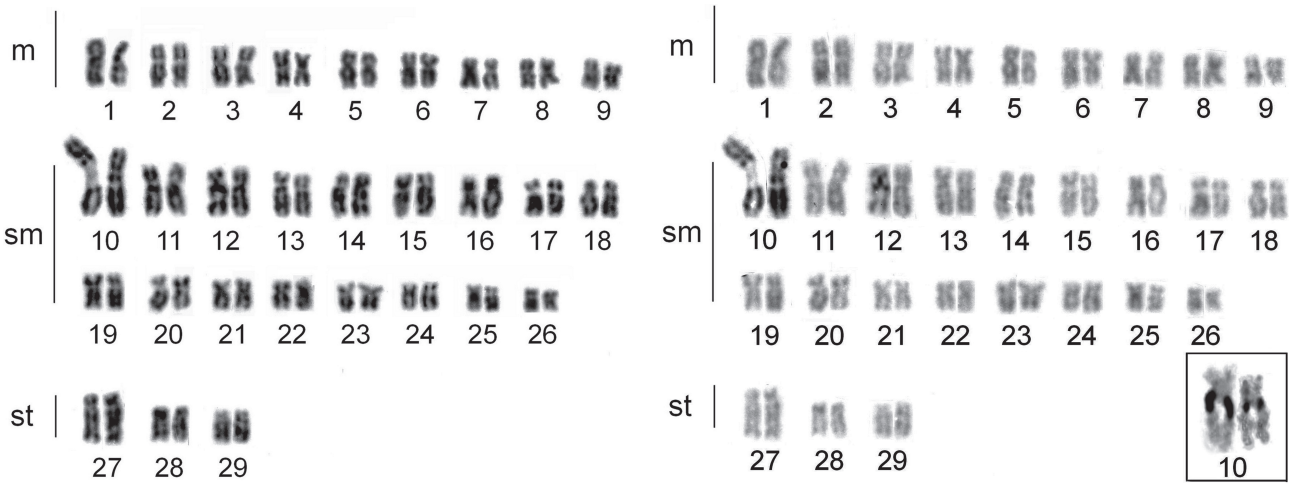
The mitotic metaphases were obtained according to Bertollo *et al.* (1978) and Foresti *et al.* (1993). Chromosome morphology was determined according to the arm size relation proposed by Levan *et al.* (1964). The fundamental number (FN) was established through the sum of the number of chromosome arms, 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* Hibridization (FISH) technique (Pinkel *et al.* 1986), with 77% stringency and probes obtained from *Prochilodus argenteus* Spix & Agassiz, 1829 (Hatanaka & 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.

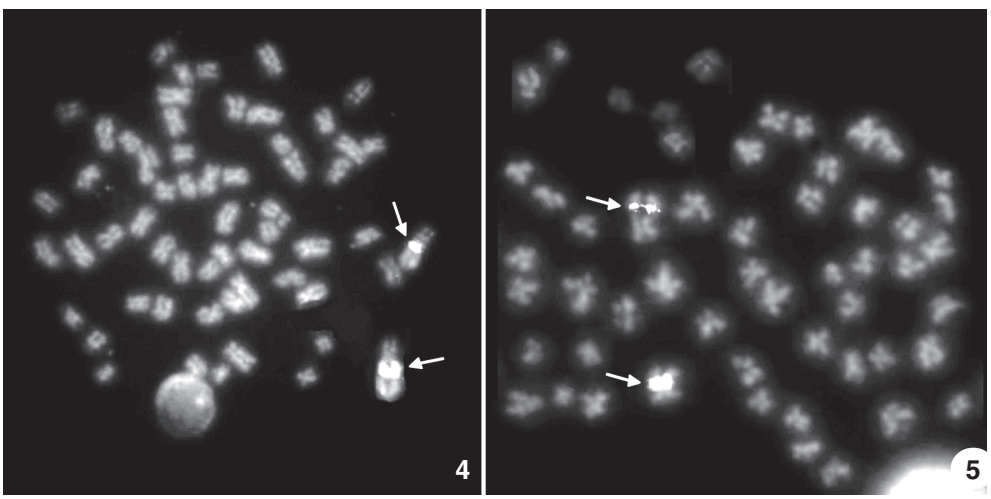
RESULTS

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).



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.

DISCUSSION

The 2n = 58 karyotype identified in *I. 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 *I. schubarti*, is the most common in the genus, being present also in *I. piperatus* (Vissotto *et al.* 2001), *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

Table I. Cytogenetic data avaliable for *Imparfinis* spp.

Species	Locality, State	2n	NF	Karyotype	NOR	References
I. hollandi	Iguaçu river, PR	42	84	22m+10sm+10st	pair 18 – terminal – st	Margarido & Moreira-Filho (2008)
Imparfinis borodini *	Quinta stream, SP	52	116	22m+26sm+4st	two pairs – terminal	Vissotto *et al.* (1999)
I. piperatus cyt. A	Araras river, SP	58	116	32m+26sm	pair 1 – interstitial – m	Vissotto *et al.* (2001)
I. piperatus cyt. B	Grande river, SP	58	106	26m+22sm+8st+2a	pair 1 – interstitial – m	Vissotto *et al.* (2001)
Imparfinis cf. *piperatus*	Juquiá river, SP	56	108	22m+26sm+4st+4a	pair 28 – interstitial – a	Vissotto *et al.* (2001)
Imparfinis cf. *piperatus*	Juquiá river, SP	56	112	24m+12sm+20st	pair 22 – interstitial – st	Fenocchio *et al.* (2003)
I. mirini	Jacutinga stream, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto *et al.* (1997)
I. mirini	Jacutinga stream, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
I. mirini	Alambarí river, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
I. mirini	Capivari river, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
I. mirini	Três Barras stream, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
I. mirini	Quinta stream, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto *et al.* (1997)
I. mirini	Quinta stream, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
I. mirini	Novo river – SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
I. mirini	Pardo river, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
I. mirini	Canta Galo stream, SP	58	116	24m+34sm	pair 1 – interstitial – m	Vissotto (2000)
Imparfinis aff. *I. schubarti*	Canta Galo stream, SP	58	108	22m+18sm+10st+8a	pair 1 – interstitial – m	Fenocchio *et al.* (2003)
Imparfinis aff. *I. schubarti*	Ribeirão Três Bocas, PR	58	108	22m+18sm+10st+8a	pair 1 – interstitial – m	Fenocchio *et al.* (2003)
Imparfinis aff. *I. schubarti*	Tigabi river, PR	58	116	28m+28sm+2st	pair 1 – interstitial – m	Stolf *et al.* (2004)
Imparfinis schubarti	Ribeirão das Minhocas, MG	58	116	18m+34sm+6st	pair 10 – interstitial -sm	Present Study

(SP) São Paulo, (PR) Paraná, (MG) Minas Gerais. * Reported as *H. longicauda*.

nucleolar organizing region from an interstitial to a terminal position may represent a synapomorphy further grouping *I. hollandi* and *I. borodini*.

According to Fenocchio *et al*. (2003), *Cetopsorhamdia* sp. collected in the River Canta Galo (city of Itirapina, SP, Brazil) has a simple NOR located in the largest pair of metacentric chromosomes and 2n = 58. *Cetopsorhamdia iheringi* Schubart & Gomes, 1959 (Vissotto *et al*. 1999) also has 2n = 58 and simple interstitial Ag-NOR in the largest chromosome pair, although that pair is submetacentric. In this last study, a conspicuous heterochromatic block adjacent to the Ag-NOR was also observed. Within the Heptapteridae, both *Imparfinis* and *Cetopsorhamdia* belong to the "*Nemuroglanis* subclade" (Ferraris 1988, Lundberg *et al*. 1991a, Bockmann 1994). The absence of these characteristics in heptapterids that are not members of the subclade, such as *Rhamdia* Bleeker, 1858 and *Pimelodella* Eigenmann & Eigenmann, 1888, suggests that they may be apomorphic characters shared at least by these two genera.

The use of the 18S rDNA probe confirmed the data obtained with the silver nitrate staining and revealed heteromorphism in the single NOR-bearing chromosome pair in *I. schubarti*. Stolf *et al*. (2004) identified in *Imparfinis* sp. aff. *I. schubarti* a pattern identical to the one observed in this study, however with the absence of heteromorphism.

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 synthenic 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 & Guazzelli 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-

netic 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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