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Cytogenetic Studies in Three *Pimelodella meeki* Populations (Pisces, Pimelodidae) from Tibagi River Basin (Brazil)

A. P. VIDOTTO, A. C. SWARÇA, A. S. FENOCCHIO, AND A. L. DIAS

From the Universidade Estadual de Londrina, Depto. de Biologia Geral, Londrina, PR, Brazil, 86051–970, Caixa Postal 6001 (Vidotto, Swarca, and Dias), and Depto. de Genética, Universidad Nacional de Misiones, Posadas, Misiones, Argentina (Fenocchio).

Address correspondence to A. L. Dias at the address above, or email: anadias@uel.br.

We analyzed cytogenetically specimens of *Pimelodella meeki* from Tibagi River at Limoeiro (LM) and from two tributaries, Couro do Boi (CB) and Gabriel da Cunha (GC) Rivers. All specimens presented $2n = 46$ chromosomes, which were the karyotypes composed by 15 pairs metacentric (M) + 6 pairs submetacentric (SM) + 2 pairs subtelocentric (ST). In specimens of GC, CB, and LM, the results of analyses of the nucleolus organizer regions (NORs), done by means of $AgNO_3$ and CMA_3 staining, showed that they are identical, located in terminal position on the short arm of a SM chromosome pair, and they were observed to be a size heteromorphism in some metaphase plates. FISH with 18S rDNA probe yielded evidence for these regions but not for the size variation, indicating that they are not due to a greater number of NOR cistrons in one of the homologue chromosomes. An interesting characteristic of these regions is that they could appear divided in blocks, as evidenced by all the techniques. This work makes clear the necessity for more deeply systematic studies, because of the confused taxonomic situation of the genus *Pimelodella*.

Pimelodella is one of the more diverse genera in the family Pimelodidae, with approximately 60 species distributed from the La Plata River basin, in the south of South America up to Panama, in Central America (Burgess 1989). In the Tibagi River basin (Parana State, Brazil), the order Siluriformes, with 22 species belonging to seven families, represents around 23% of the total abundance of the fish fauna, and 86% correspond to fishes of the Pimelodidae family (Bennemann et al. 1995). Currently, in this basin have been collected two different species of *Pimelodella*, *P.* aff. *avanhandavae* and *P.* aff. *meeki* (Shibatta et al. 2002.)

Few cytogenetic studies have been done in this genus; however, studies have demonstrated extensive karyotypic variability. Genus *Pimelodella* presents three diploid numbers: $2n = 46$ in *Pimelodella* sp. (Dias and Foresti 1993; Toledo and Ferrari 1976; Vasconcelos and Martins-Santos 2000; Vissotto et al. 1999) and *Pimelodella notomelas* (Vidotto 2001); $2n = 52$ in *Pimelodella* sp2. (Vasconcelos and Martins-Santos 2000), *Pimelodella cristata* (Faria AA et al. unpublished data), and *Pimelodella* aff. *avanhandavae* (Swarça et al. 2003); and $2n = 58$ in *Pimelodella transitoria* and *Pimelodella kronei*, both analyzed by Almeida-Toledo et al. (1992).

Because of difficulty in correctly identifying the species of *Pimelodella*, some taxonomic mistakes can occur, attributing determined cytogenetic characteristics to ambiguous taxonomic entities.

The objective of the present study was to characterize cytogenetically different populations of *Pimelodella meeki* from the Tibagi River basin, Parana, Brazil, and to analyze the NORs by means of the $AgNO_3$, CMA_3, and in situ hybridization (FISH) with 18S rDNA probe.

Materials and Methods

We analyzed cytogenetically 44 specimens of *Pimelodella meeki*. Four specimens (1 male and 3 females) from Tibagi River at Limoeiro (LM) and 40 from two tributaries: 17 specimens (9 males and 8 females) from Couro do Boi River (CB), and 23 (16 males, 5 females, and 2 of undetermined sex) from Gabriel da Cunha River (GC). Mitotic chromosome preparations were obtained according to Bertollo et al. (1978). Chromosome morphology was determined on the basis of arm ratios as proposed by Levan et al. (1964). Metacentric (M), submetacentric (SM), and subtelocentric (ST) chromosomes were considered as bi-armed. NOR silver staining was performed according to the method of Howell and Black (1980), and chromomycin (CMA_3) staining was done as described by Verma and Babu (1995). The 18S rDNA segment containing 1700pb of the fish *Oreochromis niloticus* was used for fluorescence in situ hybridization (FISH) and labeled with biotin-14-dATP by nick translation (Gibco cat N° 18247-015), according to the manufacturer's

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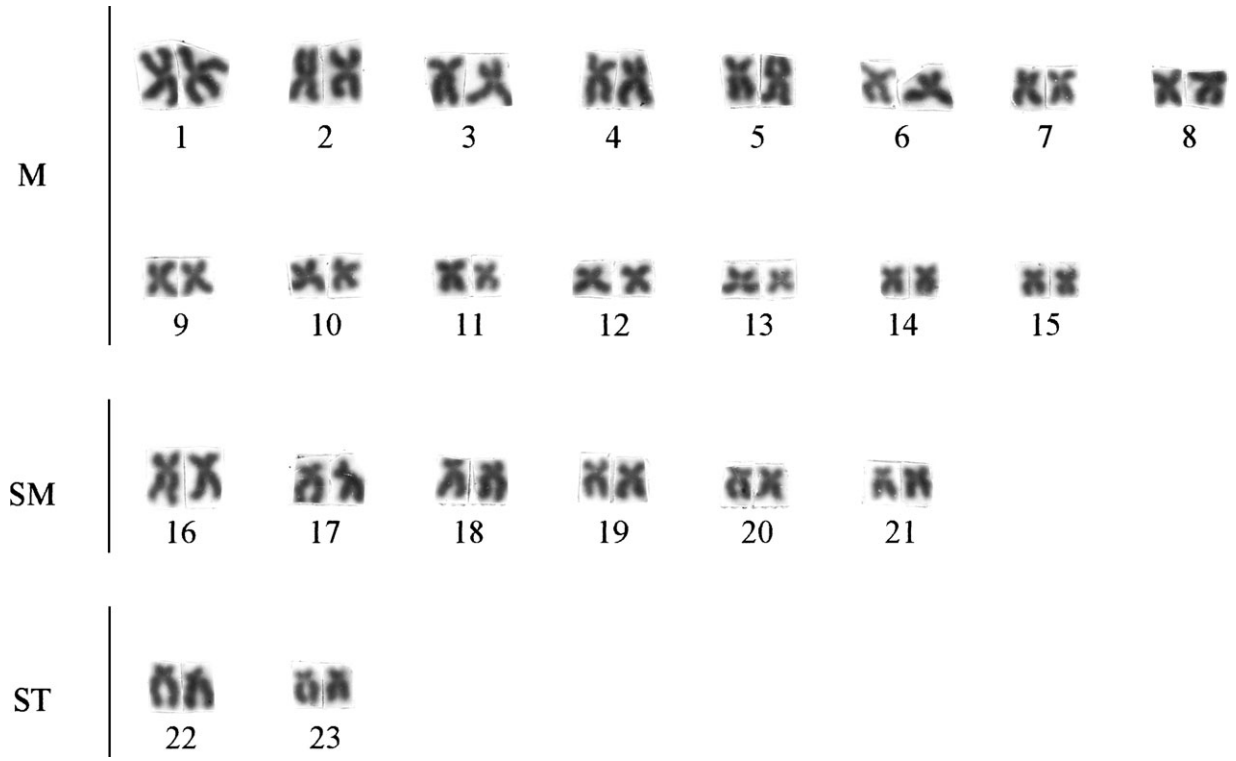

Figure 1. Giemsa-stained karyotype of *P. meeki* from LM.

instructions. The hybridization technique, posthybridization washes, and visualization were carried out as reported by Swarça et al. (2001). These techniques were applied to study the location, composition, and eventual polymorphisms of the nucleolar organizers (NORs).

Results

Karyotypes of *Pimelodella meeki* from all populations analyzed here were constituted by $2n = 46$ chromosomes and composed of 30 M, 12 SM, and 4 ST, and NF = 92 (Figure 1).

In specimens of GC, CB, and LM, the AgNORs were located at terminal regions of the short arm of an SM pair, in which was observed a size heteromorphism between homologue chromosomes (Figure 2a). However, in some metaphases of CB population we observed separated AgNOR blocks (Figure 2d).

In *P. meeki* populations, staining with CMA_3 evidenced bright signals coincident with the AgNORs. Additionally, CMA_3 evidenced the same NOR heteromorphism (Figure 2b) and segmentation of NORs as was observed in the sample of CB by means of silver staining (Figure 2d).

The FISH technique was employed to localize 18S rDNA in the chromosomes of all populations. The FITC signals appeared on one single chromosome pair, and no detectable polymorphism was observed between homologue chromosomes (Figure 2c). The FISH technique also evidenced separate fluorescent blocks in specimens from CB (Figure 2d).

Discussion

P. meeki analyzed here had $2n = 46$ chromosomes, the karyotypes of all three populations (LM, GC, and CB) being identical, with 15 pairs of M, 6 pairs of SM, and 2 pairs of ST chromosomes, showing an FN of 92 (Figure 1).

The diploid number of 46 chromosomes is found in several species and populations of *Pimelodella*, such as *Pimelodella* sp. from the Mogi-Guaçu and Pardo Rivers (Dias and Foresti 1993; Toledo and Ferrari 1976), *P. avanhandavae* from the Araquá and Capivara Rivers (Vissotto et al. 1999), and *Pimelodella* sp1. from the Paraná River (Vasconcelos and Martins-Santos 2000). All these populations, with the exception of *Pimelodella* sp. studied by Toledo and Ferrari (1976), which had 38 M-SM and 8 ST-A chromosomes, had 40 M-SM and 6 ST-A chromosomes. The populations of *P. meeki* examined here possess a different karyotype from those cited. These variations could be due to condensation differences within or between cells, or could be a result of chromosome rearrangements.

In *P. meeki* from the GC and CB Rivers and from LM, the AgNOR was observed on the short arm of one pair of SM chromosomes, with a size heteromorphism of this region (Figure 2a). Examples of heteromorphic AgNORs in this genus were observed in *Pimelodella* sp1. and *Pimelodella* sp2. (Vasconcelos and Martins-Santos 2000), *Pimelodella* sp. from the Mogi-Guaçu River as described by Dias and Foresti (1993), *P. avanhandavae* (Vissotto et al. 1999), and *P.* aff. *avanhandavae* (Swarça et al. 2003).

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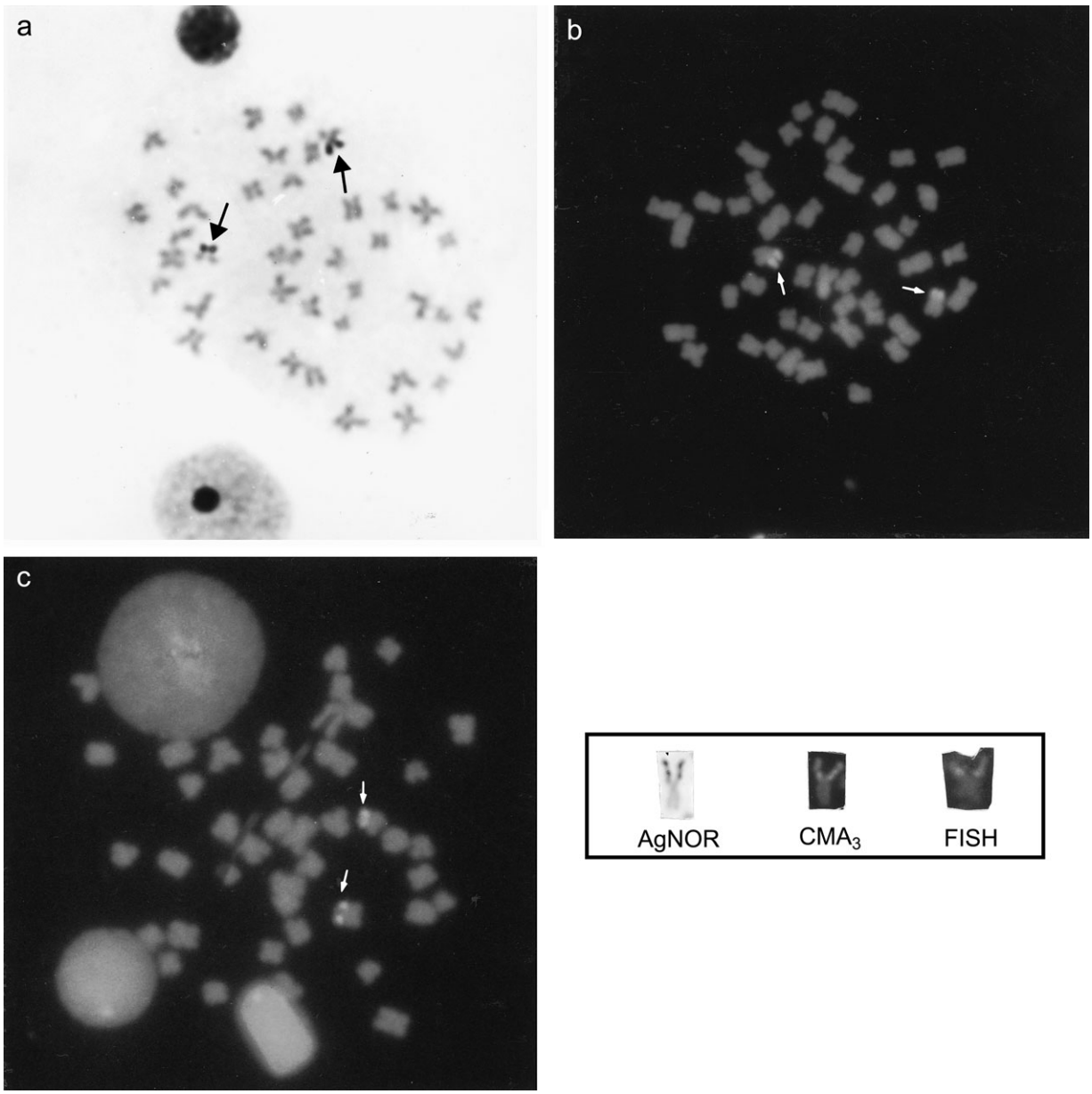

Figure 2. Somatic metaphases of *P. meeki*: **(a)** AgNOR from LM, **(b)** CMA_3 from LM, **(c)** FISH with 18S rDNA probe from LM, and **(d)** NOR chromosome pairs from CB with separated blocks after AgNOR, CMA_3 and FISH. The arrows show the nucleolar chromosomes.

Treatment with the fluorochrome CMA_3 in all populations showed a correspondence with the AgNOR staining, evidencing the same size heteromorphism of NOR (Figure 2b). This feature is very common in pimelodids (Swarça et al. 2001, 2003) and reflects the richness in CG base pairs of this region.

In situ hybridization with a 18S rDNA probe in *P. meeki* showed one pair of chromosomes without differences in the size of the signals between homologues (Figure 2c). The heteromorphisms evidenced by $AgNO_3$ and CMA_3 staining probably have a functional origin, meaning that they are due to differences in the expression of ribosomal genes or even to heterochromatin accumulation, and not related to a greater number of rDNA gene copies on one of the homologues. This result was also observed by Viñas et al. (1996) in some fish species and by Swarça et al. (2003) in *P.* aff. *avanhandavae*. In CB specimens it was possible to visualize the FITC signals as discrete blocks, confirming again the results obtained by means of AgNOR and CMA_3(Figure 2d).

Present results add new information toward a better characterization of *Pimelodella* genus and evince the confused taxonomy of this fish group, on which several authors have

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reported with uncertain classifications due to a lack of definition of specific names. It could be that the same species has been studied under different denominations, or vice-versa.

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