Cytogenetic Studies in Two Species of Genus *Pimelodella* (Teleostei, Siluriformes, Heptapteridae) from Iguatemi River Basin, Brazil

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Summary *Pimelodella* is one of the genera belonging to the family Heptapteridae consisting of endemic neotropical fishes; it has shown a wide variability in karyotype. The present study aimed to evaluate cytogenetically two species belonging to the genus *Pimelodella* from Iguatemi River Basin, MS, Brazil. In the specimens of *P. avanhandavae*, the diploid number was $2n=52$ chromosomes, distributed in $24m+20sm+08st$, with multiple NORs systems. The heterochromatin was weakly visualized with C-banding in telomeric and/or centromeric regions of a few chromosomes. For the specimens of *P. gracilis*, the diploid number was $2n=46$ chromosomes, distributed in $20m+18sm+06st+2a$ to with a simple NORs system, and heterochromatic blocks in the centromeric and telomeric regions, with variable intensity of staining, and conspicuous interstitial blocks. The two species of *Pimelodella* differ in diploid number and karyotypic formula, indicating that chromosomal rearrangements, such as fissions and/or centric fusions, may have occurred during the diversification of these two species.

Key words *Pimelodella avanhandavae*, *Pimelodella gracilis*, Chromosomes, Chromosomal rearrangements, Karyotypic evolution.

Pimelodella is one of the genera belonging to the family Heptapteridae consisting of endemic neotropical fishes. It has a wide distribution in streams of Central and South America, and several representatives of specific locations in areas of endemism have been recognized ichthyologically (Bockmann and Guazelli 2003). It includes fish both small and medium sized, representing one of the largest radiations of catfishes (de Pinna 1998).

Despite recently being elevated to the status of family, Heptapteridae, which until recently were considered a subfamily of Pimelodidae, cytogenetic studies have since 1976, first was in *Rhamdia hilarii* and *Pimelodella* sp. However, of the 26 genera belonging to the family Heptapteridae, only seven genera have been studied cytogenetically, and of 200 species only 17 species are characterized cytogenetically (Borba 2009). Existing cytogenetic reports on the genus *Pimelodella* show great variability in karyotype, with diploid numbers ranging from $2n=46$ in *Pimelodella* sp. (Vasconcelos and Martins-Santos 2000, Vissoto *et al.* 1999) to $2n=58$ in *P. kronei* (Almeida-Toledo *et al.* 1992).

The genus *Pimelodella* is characterized by a karyotype mainly formed by pairs of metacentric and submetacentric chromosomes, with subtelocentric and acrocentric chromosomes occurs the least, while maintaining a high number of fundamental arms, ranging between 80 and 116 (Swarça

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et al. 2003). Sex chromosomes systems of type XX/XY were described by Dias and Giuliano-Caetano (2002) in *Pimelodella* sp. and more recently been found in *P. boshimai* as a sexual system of the type $X^1X^1X^2X^2/X^1Y^1X^2Y^2$ (Garcia 2005).

Considering the absence of cytogenetic information on the *Pimelodella* genus from the Iguatemi River basin and the broad karyotype variation found in this genus, this study is an attempt to conduct a chromosome characterization of *P. avanhandavae* and *P. gracilis* in order to contribute new cytogenetic data on this group.

Materials and methods

P. avanhandavae (7 males, 5 female, and 3 indeterminate sex) and *P. gracilis* (4 males, 4 female, and 4 indeterminate sex) were collected from the Água Boa stream (23°50'16, 65"S and 54°20'55", 54"W) a tributary of the Iguatemi River (Mundo Novo, MS, Brazil).

The fishes were identified and deposited in the State University of Mato Grosso do Sul, unit Mundo Novo. Before being eviscerated to obtain the chromosomes, the fishes were anesthetized by an overdose of clove oil (Griffiths 2000). Metaphase chromosomes were obtained from anterior kidney cells using the air-drying technique (Bertollo *et al.* 1978). Analysis of the C-positive heterochromatin (C-bands) followed the basic procedure of Sumner (1972), with some minor adaptations. The NORs were detected by means of silver nitrate staining (Ag-NORs), according to Howell and Black (1980).

About 30 metaphases were analyzed for each specimen and those with a better quality were employed for karyotype analysis. The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a) according to their arm ratio (Levan *et al.* 1964). For the determination of the fundamental number (FN), or number of chromosome arms, the m, sm, and st chromosomes were considered as bearing two arms and the acrocentric chromosomes only one arm.

Results

P. avanhandavae presented a modal diploid number of $2n=52$ chromosomes in males and females, distributed in $24m+20sm+08st$, with a FN of 104, with no morphological chromosome difference between the sexes (Fig. 1A). In *P. gracilis*, the diploid number was $2n=46$ chromosomes, distributed in $20m+18sm+06st+2a$, with a FN of 90, with no morphological chromosome difference between the sexes (Fig. 1B).

The Ag-NORs were located in a telomeric position on the short arm of one subtelocentric pair and in a telomeric position on the long arm of one acrocentric pair in *P. avanhandavae* (Fig. 2A), and were located in a telomeric position on the short arm of one metacentric chromosome in *P. gracilis* (Fig. 2B).

In *P. avanhandavae*, the heterochromatin was weakly visualized with C-banding in the telomeric and/or centromeric regions of a few chromosomes (Fig. 2C). While in *P. gracilis*, heterochromatic blocks were observed at the centromeric and telomeric regions of a few chromosomes, with variable intensity of staining, and conspicuous heterochromatic blocks were observed at pericentromeric regions in 1 submetacentric pair (Fig. 2D).

Discussion

The present diploid number obtained in the species *P. avanhandavae* corroborates with those previously reported for *P. avanhandavae* (Swarça *et al.* 2003). However, distinct karyotypic formulae were observed, principally in relation to subtelocentric chromosomes found in the present study,

Fig. 1. Conventional Giemsa-stained karyotypes of (a) *Pimelodella avanhandavae* and (b) *Pimelodella gracilis*.

which were not described for the populations previously evaluated. In contrast to *P. avanhandavae*, the present population of *P. gracilis* showed a different diploid number, namely $2n=46$ chromosomes; this number of chromosomes had been found in *Pimelodella* sp. (Dias and Foresti 1993) and *P. meeki* (Vidotto *et al.* 2004). Although the diploid number is equal to these three species of *Pimelodella*, their karyotypic constitutions are different, with an FN of 90 for the *P. gracilis* analyzed here and FNs of 92 for both *Pimelodella* sp. and *P. meeki* (Dias and Foresti 1993, Vidotto *et al.* 2004). In the *Pimelodella* species, the numerical maintenance coupled with variation in karyotypic formulae could represent the occurrence of structural rearrangements, such as inversion and/or translocation. On the other hand, in the *Pimelodella* species, as seen in *P. avanhandavae* and *P. gracilis*, diploid numbers differ, indicating that chromosomal rearrangements, such as fissions and/or centric fusions, may have occurred during the diversification of these two species. Therefore, the results obtained suggest that the species of the genus *Pimelodella* have undergone divergent karyotypic evolution.

By silver nitrate staining, the *P. avanhandavae* specimens here analyzed presented multiple NORs, while a single pair was detected in the population of *P. avanhandavae* from Tibagi River (Swarça *et al.* 2003) after it was analyzed for Ag-NOR, CMA₃ and rDNA-18S, suggesting different numbers of rDNA cistrons among individuals. Simple NORs were also detected in the present study in specimens of *P. gracilis*, as has previously been seen in *P. gracilis* (Garcia and Almeida-Toledo 2010), *P. boshimai* (Roman and Margarido 2002) and *P. kronei* (Almeida-Toledo *et al.* 1992). A single chromosome pair bearing NORs is a common feature shared by several Heptapteridae (Swarça *et al.* 2003, Stolf *et al.* 2004).

The C-banding revealed a small amount of constitutive heterochromatin in the studied species; these results were also similar to those described previously for other populations of *Pimelodella* (Swarça *et al.* 2003, Garcia and Almeida-Toledo 2010). Furthermore, in *P. avanhandavae*, the

Fig. 2. Ag-NORs of (a) *Pimelodella avanhandavae* and (b) *Pimelodella gracilis*. Distribution pattern of constitutive heterochromatin in (c) *Pimelodella avanhandavae* and (d) *Pimelodella gracilis*. The arrows indicate the sites of Ag-NORs and arrowheads indicate interstitial heterochromatic blocks.

presence of metacentric pairs with conspicuous bi-telomeric C-bands, as seen in previous studies in this species (Swarça *et al.* 2003), was not detected. In *P. gracilis*, only one chromosomal pair bore interstitial C-bands, while *P. gracialis* presented two chromosomal pairs bearing interstitial C-bands (Garcia and Almeida-Toledo 2010). The occurrence of interstitial heterochromatic blocks in *Pimelodella* allows the differentiation of species and populations.

The present paper showed that *P. avanhandavae* and *P. gracilis*, despite morphologically being very similar, cytogenetically can easily be separated by differences in diploid number and the presence of interstitial heterochromatic blocks. These results are testament to the fact that cytogenetics can help to clarify taxonomic identification.

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