

## Cytogenetic analysis on *Pterophyllum scalare* (Perciformes, Cichlidae) from Jari River, Pará state.

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**Abstract** — Cytogenetic studies were carried out on eighteen wild specimens of *Pterophyllum scalare* from Jari River, in Pará state, and the results were compared to literature. Mitotic chromosomes were obtained from kidney cells and the analysis was done using: C-banding, Ag-NOR staining, Chromomycin A<sub>3</sub>/DAPI sequential staining and fluorochrome *in situ* hybridization with human telomere probes. All individuals showed a chromosome number of  $2n = 48$  (12 M/SM and 36 ST/A) and  $FN = 60$ . No differences were detected between male and female karyotypes, indicating the absence of morphologically differentiated mitotic sexual chromosomes. Constitutive heterochromatin blocks were located at the centromeric and pericentromeric regions of all chromosomes. The largest submetacentric pair showed a differential staining on their short arms. Only two NOR bearing chromosomes were detected, and the stainings were observed at the distal region of the short arm of the largest chromosome pair, matching the secondary constriction. Chromomycin A<sub>3</sub> stained the NOR and the centromeres of some chromosomes. DAPI-bands were observed at the centromeric regions of all chromosomes. Telomere sequences hybridised only at the terminal regions.

**Key words:** banding, chromosome, DAPI, Fish, ornamental

### INTRODUCTION

Cichlids show a huge form, colour and size diversity (STIASSNY 1991), comprising 227 genera and 1,292 species distributed throughout Africa, South and Central America and southern North America, Madagascar, Southern India and Sri Lanka (NELSON 1994; KULLANDER 1998). There are more than one hundred cichlid species in the Amazon basin (FERREIRA *et al.* 1998), which are of great importance both as protein sources and as ornamental fish. Among the Perciformes, the family Cichlidae has the largest number of cytogenetically analysed species, which do not even represent 10% of all valid species (BRUM 1995). So far, 135 species (106 from the New World and 29 from the Old World) had their karyotypes analysed, and many papers only describe the haploid/

diploid and fundamental numbers. The number of cichlid species examined through banding techniques is still very scarce (FELDBERG *et al.* 2003). Amongst the most striking chromosomal characteristics in this family stands out a tendency for maintaining the diploid number as 48, karyotypes constituted mostly by subtelo-acrocentric chromosomes, single NORs on the largest chromosomal pair and constitutive heterochromatin present mainly at the centromeric region of all chromosomes (THOMPSON 1979; FELDBERG & BERTOLLO 1985a; b; MARTINS *et al.* 1995; FELDBERG *et al.* 2003).

The subfamily Cichlasomatinae (family Cichlidae) is divided into three tribes (Acaroniini, Heroiini and Cichlasomatini). The tribe Heroiini has around 100 species in Central and 40 in South America (KULLANDER 1998). The genus *Pterophyllum* Heckel, 1940 belongs to this tribe and has three species: *P. scalare* (Lichtenstein, 1823), *P. altum* Pellegrin, 1903 and *P. leopoldi* (Gosse, 1963). It is endemic to South America, being

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widely distributed in the Amazon basin (AXELROD 1995). They are known as angelfishes.

*Pterophyllum scalare* is one of the best known and popular Amazonian ornamental fish species in ornamental fish trade and aquaculture (YAMAMOTO *et al.* 1999). The international trade has shown a great interest for Amazonian species belonging to the family Cichlidae, and its demand is becoming increasingly higher (CHAO 1993).

Of the three species of the genus *Pterophyllum*, only *P. scalare* was cytogenetically analysed. POST (1965) and SCHEEL (1973) only determined the haploid number of 24, whereas THOMPSON (1979) and KRICHANA *et al.* (1996), who described the karyotype of specimens obtained from commercial sources, found the same data:  $2n = 48$ , being 4 meta-submetacentric (M/SM) and 44 subtelocentric (ST/A) and fundamental number (FN) equal to 52. KRICHANA *et al.* (1996) still reported a single NOR for this species, with stainings on the distal regions of the short arms of one pair of subtelocentric chromosomes.

This paper provides the description of karyotype of *P. scalare*, collected in Jari River-PA, through classical and molecular cytogenetic techniques. It adds new information to existing cytogenetic data for cichlids, providing further information that may contribute for them taxonomy, evolution and phylogeny.

## MATERIAL AND METHODS

Eighteen individuals (seven males, eight females and three of undetermined gender) of the

species *Pterophyllum scalare* from Jari River ( $00^{\circ} 50'46.2''\text{S}$  and  $52^{\circ} 29'22.3''\text{W}$ ), Monte Dourado, Pará, Brazil, were analysed.

Mitotic chromosomes were obtained from kidney cells through the direct extraction technique with slight changes adapted by BERTOLLO *et al.* (1978) for cytogenetic studies in fish. Chromosomal analysis was carried out using the best preparations and the diploid number was established after counting up to 50 metaphases of each individual. Chromosomes were measured and classified into two categories (M/SM and ST/A) according to the limits proposed by LEVAN *et al.* (1964) for the ratio between the arms. For determining FN, the chromosomes in M/SM were considered to have two arms and those in ST/A only one. Karyotypes were mounted by arranging chromosomes in pairs and size decreasing order within their respective morphological categories.

Chromosomal preparations were submitted to C-banding techniques (SUMNER 1972), Ag-NOR staining (HOWELL & BLACK 1980), Chromomycin A<sub>3</sub> and (SCHWEIZER 1980), DAPI staining (PIEZARKA *et al.* 2005) and *in situ* hybridization with human telomeric probes (*Oncor*, "All Human Telomere Probe", code P5091) according to the recommended protocol.

## RESULTS

The specimens presented  $2n = 48$ , with 12 M/SM and 36 ST/A chromosomes and  $NF = 60$  (Figure 1). A noted trait was the heteromorphic secondary constriction at the short arm distal re-

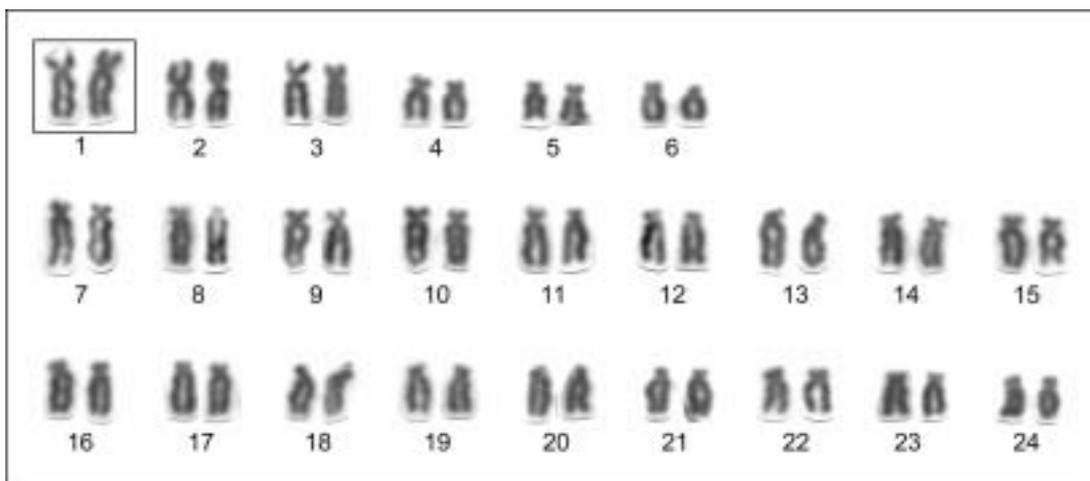


Fig. 1 — Giemsa stained karyotype of a *P. scalare*. The boxed chromosome pair is the NOR bearer. Six pairs were metacentric or submetacentric (M-SM, upper row) and 18 pairs were subtelocentric or acrocentric (ST-A, lower rows).

gion of the biggest chromosomal pair of the karyotype. No differentiated mitotic sexual chromosomes were detected on this sample.

Large and evident constitutive heterochromatin blocks were observed at the centromeric and pericentromeric regions of all chromosomes (Figure 2). The pair 1 also presented a heterochromatic block with a less intense staining than that of the centromere. That block covers almost the whole short arm.



Fig. 2 — C-banding pattern of *P. scalare*. The arrows show the pair 1. See text for details.

NORs are located in the secondary constriction, also presenting a pronounced size heteromorphism (Figure 3). Ag-NOR/C-banding sequential staining showed that the heterochromatic block observed on the NOR bearer chromosome short arm lies adjacent but not coincident with this region (Figure 4).

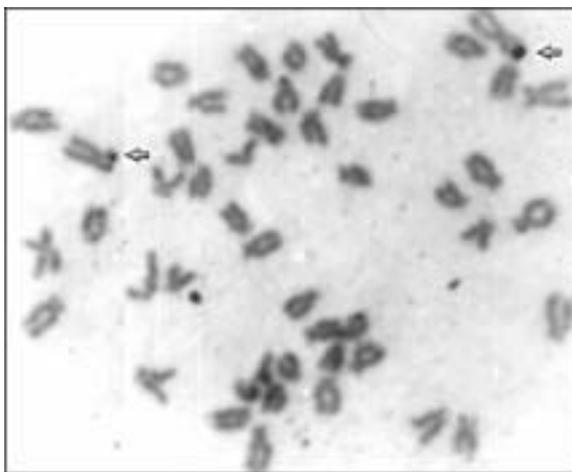


Fig. 3 — NOR-staining pattern of *P. scalare*. The arrows show the pair NOR region. See text for details.

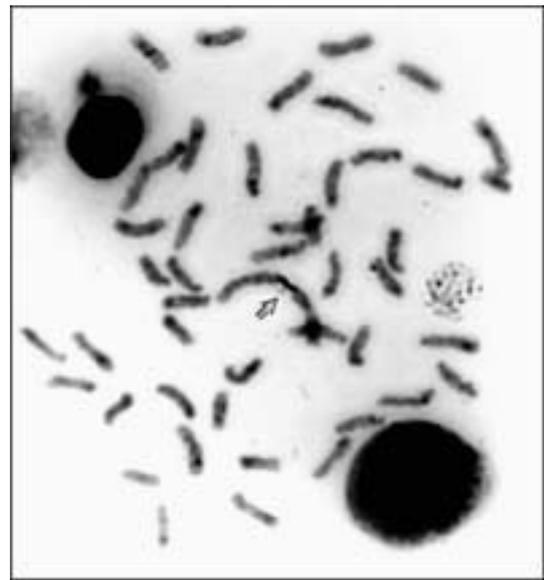


Fig. 4 — Sequential Ag-NOR staining / C-banding of *P. scalare*. It showed that the heterochromatic block observed on the NOR bearer chromosome short arm lies adjacent but not coincident with this region (arrow).

The most conspicuous CMA<sub>3</sub>-bands corresponded to Ag-NORs. Guanine-cytosine (GC) rich heterochromatin bands with fluorescence intensities slightly lower than those of the NORs also were produced by CMA<sub>3</sub> staining in some chromosomes (Figure 5a). DAPI-bands were observed at the centromeric regions of all chromosomes, including at those which were stained by CMA<sub>3</sub> but not on the NOR region (Figure 5b).

Human telomeric sequences only hybridised at the chromosomes terminal regions with no interstitial staining being observed (Figure 6).

## DISCUSSION

The diploid number found for the specimens of *Pterophyllum scalare* that were analysed in this work ( $2n=48$ ) agrees with those reported in literature by THOMPSON (1979) and KRICHANA *et al.* (1996). It can be found on over 60% of the species of Neotropical cichlids (FELDBERG *et al.* 2003). The karyotypic formulae from the present study (12 M/ST + 36 ST/A) showed a clear difference relative to those in the literature (4 M/SM + 44 ST/A). However, it was similar to that obtained by Silva (personal communication, 2003) on specimens collected at Barcelos town (middle Negro River, AM).

Although other authors have not mentioned before the presence of secondary constrictions on

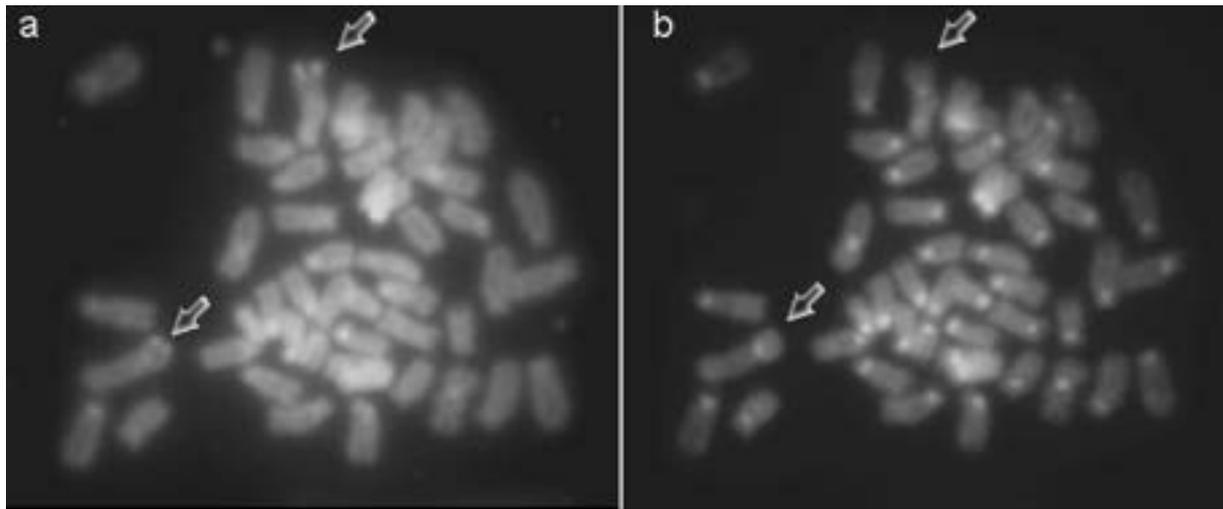


Fig. 5 — a) CMA<sub>3</sub>-banded karyotype of *P. scalare*. b) Sequenced DAPI-banded karyotype of *P. scalare*. On both figures the arrows indicate the NOR region.

the *P. scalare* karyotype, it is a common trait for the cichlid species. In general, it is strikingly pronounced on the biggest pair of the complement.

The genus *Pterophyllum* C-banding pattern is reported for the first time in the present paper, what makes it difficult to compare with data from literature. Our findings show that *P. scalare* has constitutive heterochromatin in the centromeric and pericentromeric regions of all chromosomes.

This is the pattern found for all the analysed cichlid species both from the New World and Old World (FELDBERG *et al.* 2003). However, this species presents quite large heterochromatic blocks, which is a characteristic only found on one more genus (*Symphysodon*, MESQUITA 2002) from family Cichlidae in addition to *Pterophyllum*. The largest pair appears as a good chromosomal marker, due to the presence of the heterochro-

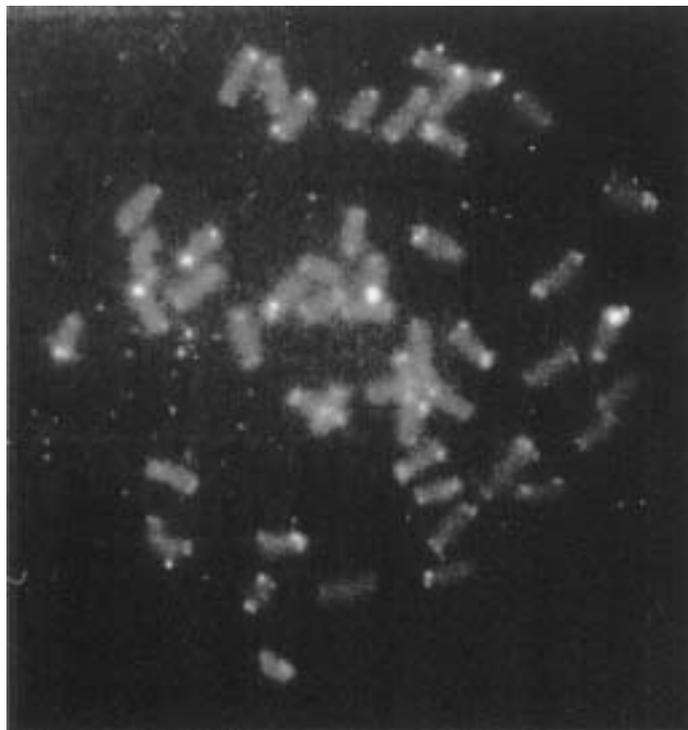


Fig. 6 — Human telomeric probes hybridized in a karyotype of *P. scalare*.

matic block on the short arm with a less intensive staining than that on the centromere.

In addition to the chromosomal formula, our findings also differed for the NORs location (ST pair, distal region on short arm) reported by KRICHANA *et al.* (1996). SILVA (personal communication) found the same NOR locations for Barcelos specimens as observed in the present paper.

Regarding to the family Cichlidae, both NOR number and position are in agreement with those in literature, since this family may be generally characterised as a fish group with system of NORs located on the largest chromosomal pairs of the karyotype (FELDBERG & BERTOLLO 1985b). According to HSU *et al.* (1975), karyotypes with only one pair of NORs may be considered as ancestors of those presenting NORs distributed in various chromosomes. Although the genus *Pterophyllum* occupies a more derivate position on the phylogenetic tree proposed by KULLANDER (1998), *P. scalare* maintains a character considered to be plesiomorphic for the family Cichlidae.

According to ALMEIDA-TOLEDO *et al.* (1985), the heteromorphism on the size of the NORs, quite frequent among fish, seems to be a general trait of this chromosomal structure. A possible explanation for the heteromorphism observed on this paper can be seen in the Figure 4 where both the NORs seem to be connected. The connection of the NORs can facilitate the occurrence of translocations between these regions (LOURENÇO *et al.* 1998). Variations on their number and position constitute important cytogenetic markers for characterizing species and populations.

The use of Chromomycin A<sub>3</sub> as a fluorescent NOR stain in fish cytogenetics in some respects is preferred over silver staining, because CMA<sub>3</sub> apparently stains DNA and will differentiate NORs regardless of previous genetic activity or chromosomal stage (AMEMIYA & GOLD 1986). On this species, two types of CMA<sub>3</sub>-bands were observed. One of these, more intense, corresponds to Ag-NORs. Another one, slightly less intense, was observed at the centromere of some chromosomes. DAPI fluorochrome binds to chromosomal regions rich in adenine-thymine (AT) base pairs and has been tried on some species of fishes (AMEMIYA & GOLD 1986). In *P. scalare*, DAPI-bands were observed at the centromeric regions of all chromosomes, showing a fluorescent pattern that was similar to that obtained by C-banding. The centromeres of some chromosomes had shown positive staining both for CMA<sub>3</sub> and DAPI, what

suggests that this centromeric heterochromatin is made of intercalated A-T and G-C rich segments.

Differences on the number of arms and location of the NORs may be accounted for chromosomal rearrangements like pericentric inversion and/or translocation, instead of heterochromatin addition, since the C-banding in our sample shows that all SM chromosomes, but for one pair, show no heterochromatic arms. Hybridization of human telomere sequence in chromosomes of animals phylogenetically distant, showed the evolutionary conservation of this DNA sequence. The lack of interstitial markers indicates that those rearrangements occurred in the karyotypic differentiation involved no telomeric regions.

The chromosomal differences of Jari (present paper) and Negro (SILVA, personal communication) Rivers individuals when compared to literature, where the sample was purchased from non specified aquarists, make it clear that there are at least two species, which might be cryptic or taxonomically mistakenly classified.

These data are very important for ornamental fish aquaculture and trade, since their management and conservation may only be achieved as long as the species are well known.

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