

Karyotypic characterization of *Prochilodus mariae*, *Semaprochilodus kneri* and *S. laticeps* (Teleostei: Prochilodontidae) from Caicara del Orinoco, Venezuela

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Fish of the family Prochilodontidae are considered one of the most important components of commercial and subsistence fishery in freshwater environments in South America. This family consists of 21 species and three genera. In the present study, the karyotypes of *Prochilodus mariae*, *Semaprochilodus kneri*, and *S. laticeps* from Caicara del Orinoco, Bolivar State, Venezuela were studied. The species *P. mariae*, *S. kneri* and *S. laticeps* exhibited $2n=54$ chromosomes (40 metacentric and 14 submetacentric), a single chromosome pair with nucleolus organizer regions, and a large amount of heterochromatin found at centromeric and pericentromeric positions in almost all chromosomes. The *P. mariae* specimens studied displayed 0 to 3 supernumerary microchromosomes. The data obtained here confirm the conservative nature of the chromosome number and morphology of Prochilodontidae and reinforce the hypothesis that small structural chromosome rearrangements were the main cause of the karyotypic diversification seen in this group.

Os peixes da família Prochilodontidae são considerados um dos componentes mais importantes da pesca comercial e de subsistência em ambientes de água doce na América do Sul. Essa família compreende 21 espécies e três gêneros. No presente estudo foram analisados os cariótipos de *Prochilodus mariae*, *Semaprochilodus kneri* e *S. laticeps* provenientes de Caicara del Orinoco, Estado Bolívar, Venezuela. As espécies *P. mariae*, *S. kneri* e *S. laticeps* apresentaram $2n=54$ cromossomos (40 metacêntricos e 14 submetacêntricos), um único par de cromossomos com regiões organizadoras de nucléolo e uma grande quantidade de heterocromatina em posição centromérica e pericentromérica de quase todos os cromossomos. Os espécimes estudados de *P. mariae* apresentaram de 0 a 3 microcromossomos supranumerários. Os dados obtidos aqui confirmam a natureza conservada do número e da morfologia cromossômica dos Prochilodontidae e reforçam a hipótese de que pequenos rearranjos estruturais foram os principais eventos fixados na diversificação cariotípica do grupo.

Key words: karyotype, C-band, NOR banding, chromosome evolution, Characiformes.

Introduction

Fish of the family Prochilodontidae are considered one of the most important components of commercial and subsistence fishery in freshwater environments in South America, with the exception of Chile, where these species are not found (Lowe-McConnell, 1975; Goulding, 1981; Vari, 1983). Castro & Vari (in press) showed that this family comprises 21 species distributed in three genera: *Ichthyoelephas* (2 species), *Prochilodus* (13 species), and *Semaprochilodus* (6 species). In Venezuela, this family is represented by two genera and

four species: *P. reticulatus*, *P. mariae*, *S. kneri*, and *S. laticeps*. The first species is found only in the Maracaibo Basin, while the other three are found in the Orinoco Basin.

Preliminary cytogenetic studies showed that *Prochilodus lineatus* (= *Prochilodus scrofa*) from the Mogi-Guaçu River, Brazil, exhibited $2n=54$ chromosomes and zero to five supernumerary microchromosomes (Pauls & Bertollo, 1983). These supernumerary microchromosomes have been widely studied under different methodologies including populational distribution, molecular constitution, meiotic behavior, and inheritance model (Oliveira *et al.*, 1997; Dias *et al.*, 1998;

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Cavallaro *et al.*, 2000; Maistro *et al.*, 2000; Jesus *et al.*, 2003). Additional studies in the genus *Prochilodus* showed that eight species also had $2n=54$ chromosomes and that the species *P. brevis* (= *P. cearensis*) had zero to two supernumerary microchromosomes with characteristics similar to those found in the supernumerary chromosomes of *P. lineatus* (Pauls & Bertollo, 1990). Cytogenetic studies conducted in species of the genus *Semaprochilodus* showed that *S. insignis* and *S. taenirus*, from the Negro River had $2n=54$ chromosomes (Feldberg *et al.*, 1987). Additionally, the species *S. taeniurus* showed a putative ZZ/ZW sex chromosome system (Feldberg *et al.*, 1987). In the present study, the karyotypes of *Prochilodus mariae*, *Semaprochilodus kneri*, and *S. laticeps*, from Venezuela are described and chromosome evolution in the family is discussed.

Materials and Methods

Three Prochilodontidae species were analyzed: *Prochilodus mariae* (21 specimens), *Semaprochilodus kneri* (24 specimens), and *S. laticeps* (7 specimens). All specimens were juveniles (immature), so sex determination was not attempted. Fish were collected with seine nets in lowland flood plains near Caicara del Orinoco, Bolivar State, Venezuela (N7°38'30", W66°09'00"). Voucher specimens were kept at the fish collection of Laboratório de Biologia de Peixes (*Prochilodus mariae* LBP 1382, *Semaprochilodus kneri* LBP 1384, and *S. laticeps* LBP 1383), Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

Chromosome preparations and staining techniques were conducted according to Foresti *et al.* (1993). Chromosome morphology was determined on the basis of arm ratio, as proposed by Levan *et al.* (1964), and chromosomes were classified as metacentric (M) or submetacentric (SM).

Results and Discussion

The species *Prochilodus mariae* exhibited $2n=54$ chromosomes, with 40 metacentric and 14 submetacentric chromosomes (Fig. 1a). The karyotype of *P. mariae* is very similar to those of other *Prochilodus* species (Pauls & Bertollo, 1990) in terms of number of metacentric and submetacentric chromosomes.

In 21 of the specimens analyzed, the modal number of supernumerary or B chromosomes found was 0 in five animals, 1 in nine animals, 2 in six animals, and 3 in one animal (Table 1), resulting in a mean number of 1.143. This value is similar to that found in a sample of *P. lineatus* collected in 1979/1980 from the natural population of the Mogi-Guaçu River that showed a B mean number equal to 1.341 (Pauls & Bertollo, 1983). An interesting result observed in the present study was the variation in the number of B chromosomes among different cells of each fish that was 17.5% (ranging from 0.00 to 44.4%), suggesting that these supernumerary chromosomes are mitotically unstable. A comparative study conducted with

the Mogi-Guaçu River population of *P. lineatus* showed that the number of cells with B chromosome numbers different from the individual modal number reduced from 44.1% in 1979-1980 (Pauls & Bertollo, 1983) to 0.9% in 1987-1989 (Cavallaro *et al.*, 2000) and 3.4% in 1991-1992 (Oliveira *et al.*, 1997). Oliveira *et al.* (1997) suggested that the variation observed in the behavior of *P. lineatus* B chromosomes is related to the fixation of a mitotically stable B chromosome set in the population of the Mogi-Guaçu River. Further analyses in *P. mariae* will be very helpful in the evaluation of the behavior of B chromosomes in this population. Another remarkable fact observed in this study was the absence of B chromosomes in only 4 animals (19%) of the sample analyzed, showing that these supernumerary chromosomes are widespread in the population of *P. mariae* studied.

Table 1. Frequency of B chromosomes among the cells analyzed of each *Prochilodus mariae* specimen. Only cells with $2n=54$ chromosomes were counted.

Specimen	Number of cells with Bs				Modal number of Bs	Cells counted
	0B	1B	2B	3B		
1	15	12	-	-	0	27
2	2	14	-	-	1	16
3	3	6	19	-	2	28
4	5	4	24	-	2	34
5	26	-	-	-	0	26
6	8	17	-	-	1	25
7	9	15	1	-	1	25
8	19	-	-	-	0	19
9	2	15	-	-	1	17
10	1	10	-	-	1	11
11	16	-	-	-	0	16
12	1	21	-	-	1	22
13	2	19	-	-	1	21
14	1	17	-	-	1	18
15	-	3	19	-	2	22
16	2	14	-	-	1	16
17	1	3	9	-	2	13
18	1	1	12	-	2	14
19	19	-	-	-	0	19
20	1	1	6	13	3	21
21	-	6	14	-	2	20

Although the presence of supernumerary chromosomes in *P. lineatus*, *P. brevis* and *P. mariae* might suggest the occurrence of a close relationship among these species, morphological studies have shown that this is not so (Castro & Vari, in press). Thus, it seems probable that B chromosomes have arisen independently in these three species. Alternatively, it may be assumed that B chromosomes were present in the ancestor of the genus and that these accessory chromosomes were lost in several species. Further data on the chromosomal constitution of other Prochilodontidae species are necessary to test these hypotheses.

A single chromosome pair with nucleolus organizer

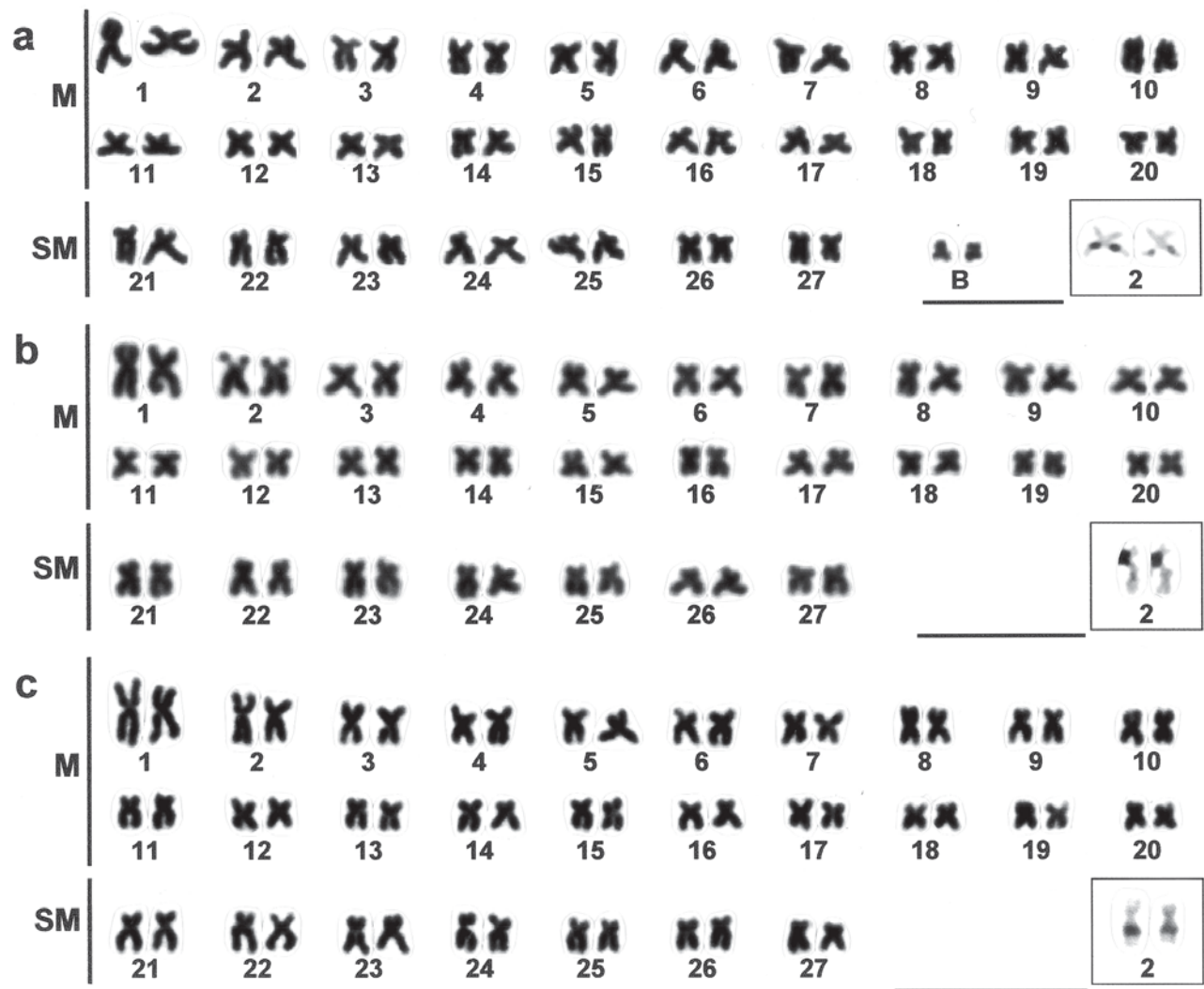


Fig. 1. Karyotypes of: a) *Prochilodus mariae* with $2n=54$ chromosomes plus two supernumerary microchromosomes (B); b) *Semaprochilodus kneri* with $2n=54$ chromosomes; and c) *Semaprochilodus laticeps* with $2n=54$ chromosomes. In the insets, silver stained chromosomes with the nucleolus organizer regions in pair 2. Bars = 10 μm .

regions (NORs), probably pair 2, was found after silver staining (Ag-NORs) in *P. mariae* (Fig. 1a). The same was reported to occur in other *Prochilodus* species (Pauls & Bertollo, 1990), showing that this characteristic is very much conserved in the genus. The remarkable conservatism of the Ag-NOR-bearing chromosome pair in *Prochilodus* species is noteworthy considering that even the species of Curimatidae (Vênere & Galetti, 1989; Feldberg *et al.*, 1992; Oliveira & Foresti, 1993; Navarrete & Júlio, 1997) and Anostomidae (Galetti Jr *et al.*, 1991a, 1991b; Galetti Jr *et al.*, 1995) that have very conservative karyotypes, usually display species-specific Ag-NOR-bearing chromosomes.

The karyotype of *P. mariae* showed a large amount of heterochromatin, basically in the centromeric and pericentromeric regions of almost all chromosomes (Fig. 2a)

as observed in *P. lineatus* (Pauls & Bertollo, 1990). Additionally, the long arm of pair 5 was entirely heterochromatic (Fig. 2a) as observed in *P. lineatus* (Maistro *et al.*, 2000). The supernumerary chromosomes of *P. mariae* were entirely heterochromatic (Fig. 2a) as observed in *P. lineatus* B chromosomes (Pauls & Bertollo, 1990; Maistro *et al.*, 2000; Jesus *et al.*, 2003).

The species *Semaprochilodus kneri* and *S. laticeps* exhibited $2n=54$ chromosomes, with 40 metacentric and 14 submetacentric chromosomes (Figs. 1b and 1c, respectively). Cytogenetic studies conducted by Feldberg *et al.* (1987) showed that *S. insignis* and *S. taeniurus* also have $2n=54$ meta-submetacentric chromosomes. The only difference found between *S. insignis* and *S. taeniurus* was the presence of a putative ZZ/ZW sex chromosome system in *S. taeniurus*

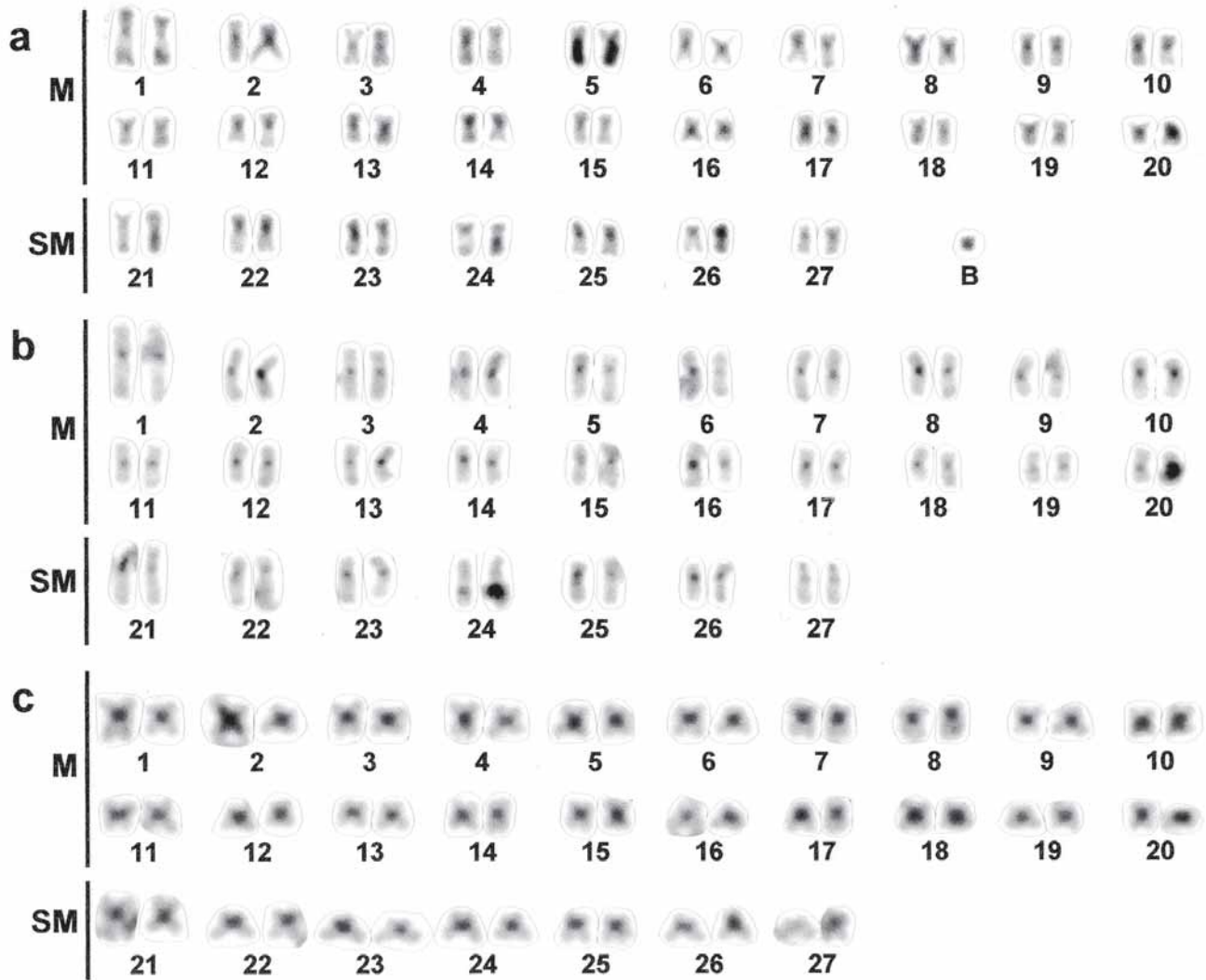


Fig. 2. Karyotypes treated with the C-banding technique. a) *Prochilodus mariae* - "B" indicates a supernumerary microchromosome; b) *Semaprochilodus kneri*; and c) *Semaprochilodus laticeps*.

(Feldberg *et al.*, 1987). The analyses of several metaphases of *S. laticeps* showed that, in some cells, the first chromosome pair was composed of two chromosomes with a small difference in size. Considering that in the present study the sex of the fish analyzed could not be precisely confirmed, these size differences and the presence of sex chromosomes in this species cannot be herein positively correlated.

In *S. kneri* and *S. laticeps*, pair 2 chromosomes clearly exhibited secondary constrictions in several metaphases corresponding to the Ag-NOR-bearing chromosomes (Figs. 1b and 1c, respectively). Both Ag-NOR-bearing chromosomes were frequently observed to associate in several *S. kneri* metaphases, which explains the occurrence of interphasic cells with one (62,5%) or two nucleolus (37,5%). Although all *Prochilodus* species exhibit NORs in the long arm of the second chromosome pair (Pauls & Bertollo, 1990; present paper), studies conducted by Feldberg *et al.* (1987) showed that *S. insignis* and *S. taeniurus* displayed interstitial Ag-

NORs in the short arm of a large submetacentric pair as observed in the present study in *S. kneri* (Fig. 1b). The presence of Ag-NORs in the long arm of the second chromosome pair in *S. laticeps* may indicate the presence of a possible ancestral condition or the occurrence of a second event of pericentric inversion in the Ag-NOR-bearing chromosomes of this species.

The karyotype of *S. kneri* and *S. laticeps* displayed a large amount of heterochromatin, mainly in the centromeric and pericentromeric regions of almost all chromosomes (Figs. 2b and 2c). An interstitial heterochromatic segment observed in the long arm of *S. kneri* chromosome pair 24 was not observed in the karyotype of *S. laticeps*. The C-banding of *S. insignis* and *S. taeniurus* was also characterized by the presence of large C-positive bands pericentromerically positioned in all chromosomes (Feldberg *et al.*, 1987). The data available show that heterochromatin distribution is also very conservative in Prochilodontidae species.

The analysis of the nucleolus organizer regions in the Prochilodontidae species herein studied showed the occurrence of polymorphisms in the Ag-NOR-bearing chromosomes of the three species, more evidently in *P. mariae* (Fig. 1a) and *S. laticeps* (Fig. 1c). This type of NOR size polymorphism was described in several *Prochilodus* species (Pauls & Bertollo, 1990) and is very common among fish (Almeida-Toledo & Foresti, 1985).

Our data confirm the conservative nature of the chromosome number and morphology of Prochilodontidae and reinforce the idea that small structural chromosome rearrangements may be the main cause of karyotypic diversification in this group (Pauls & Bertollo, 1990). The presence of $2n=54$ biarmed chromosomes have also been consistently found in the families Anostomidae (Galetti Jr *et al.*, 1991a, 1991b; Galetti Jr *et al.*, 1995), Curimatidae (Vênere & Galetti, 1989; Feldberg *et al.*, 1992; Oliveira & Foresti, 1993; Navarrete & Júlio, 1997), and Chilodontidae (Cestari *et al.*, 1990; Vênere, 1998), corroborating the hypothesis that these four families constitute a natural group in the order Characiformes (Vari, 1983).

On the other hand, small differences in chromosome morphology and Ag-NOR positioning have been observed in the karyotypes of the Prochilodontidae species, suggesting that chromosome rearrangements that do not change the number or the morphology of the chromosomes, such as paracentric inversions, may have occurred during the evolutionary process of the species, as suggested by Feldberg *et al.* (1987).

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