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Description of the karyotype of *Geophagus* cf. *proximus* (Perciformes, Cichlidae, Geophaginae)

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ABSTRACT. This study described the karyotype of *Geophagus* cf. *proximus*. Specimens were collected in Água Preta Lake, Parque Ambiental de Belém, Pará State, Brazil. The karyotype were 2n = 48 chromosomes (FN = 60: 12M/SM+36ST/A) and no sexual chromosome differentiation. C-banding showed centromeric staining in all chromosomes. The first chromosome pair, besides centromeric coloration, presented a totally heterochromatic long arm. The nucleolus organizer regions (NORs) were studied by means of $AgNO_3$. NORs were located at the short arm of the second chromosome pair.

Keywords: cytogenetic, C-banding, Ag-NOR technique, *Geophagus*.

Descrição do cariótipo de *Geophagus* cf. *proximus* (Perciformes, Cichlidae, Geophaginae)

RESUMO. Este estudo descreve o cariótipo de *Geophagus* cf. *proximus.* Espécimes foram coletados no lago Água Preta, Parque Ambiental de Belém, Estado do Pará, Brasil. O cariótipo obtido apresentou 2n = 48 cromossomos (NF = 60: 12M/SM+36ST/A), sem diferenciação de cromossomos sexuais. O bandeamento C mostrou marcações centroméricas em todos os cromossomos. O primeiro par cromossômico, além da coloração centromérica, apresentou o braço longo totalmente heterocromático. As regiões organizadoras do nucléolo (RONs) foram estudadas por meio da coloração de $AgNO_3$. As RONs foram encontradas no braço curto do segundo par de cromossomos.

Palavras-chave: citogenética, bandeamento C, técnica Ag-NOR, *Geophagus*.

Introduction

The South American cichlid genus *Geophagus* Heckel (1840) was reviewed and redescribed by Gosse (1976). This genus contains medium-sized to moderately large geophagine cichlids (TL around 12 to 30 cm), and is widely distributed in the Amazon and Orinoco basins and on the Guyana Shield. As currently known, *Geophagus* includes fourteen nominal species (LÓPEZ-FERNÁNDEZ; TAPHORN, 2004), but many others remain to be described (STAWIKOWSKI; WERNER, 2004; WEIDNER, 2000).

Previous studies on the Cichlidae family have reported a very constant diploid number 2n = 48 in different species (BENZAQUEM et al., 2008; FELDBERG; BERTOLLO, 1985; LOUREIRO, 1999; MARESCALCHI, 2005; MIZOGUCHI et al., 2007; PERAZZO et al., 2011; THOMPSON, 1979), despite a considerable variation in their fundamental number.

The Table 1 summarizes the chromosomal characteristics of several species of the subfamily Geophaginae available in the literature, including the species analyzed in the present study.

The present study aimed to present the karyotype characterization of *Geophagus* cf. *proximus* (CASTELNAU, 1855), based on conventional staining, C-banding and Ag-NOR staining.

Material and methods

For this study, four specimens (two males and two females) were collected from Água Preta Lake, Parque Ambiental de Belém (01°23'13" to 01°26'02" S and 48°23'50" to 48°26'47" W). Mitotic chromosome preparations were obtained from kidney cells using the air-drying technique of Bertollo et al. (1978). The slides were analyzed using conventional Giemsa staining, C-banding and silver staining of NOR regions.

Staining of the nucleolus organizer regions (Ag-NORs) was carried following the procedure described by Howell and Black (1980). Detection of constitutive heterochromatin (C-banding) was performed according to Sumner (1972), with some minor modifications. The modal number of the analyzed specimens was established after the analysis of 30 metaphases for each individual. Using karyotypes digitalized on Adobe Photoshop, each chromosome arm was individually measured, allowing determining

Table 1. Chromosomal characterization of the subfamily Geophaginae.

Species	2n	FN	KF	Locality	Reference
Crenicichla reticulata	48	54	6 M/SM + 42 ST/A	Uatumã river, AM, Brazil	Feldberg et al. (2004).
Crenicichla iguassuensis	48	56	8 M/SM + 40 ST/A	Salto Caxias Reservoir, PR, Brazil	Mizoguchi et al. (2007).
Apistogramma trifasciata	46	62	16 M/SM + 30 ST/A	Paraná river, Misiones, Argentina	Roncati et al. (2007).
Crenicichla lepidota	48	54	6 M/SM + 42 ST/A	Paraná river, Misiones, Argentina	Roncati et al. (2007).
Gymnogeophagus balzanii	48	50	2 M/SM + 46 ST/A	Paraná river, Misiones, Argentina	Roncati et al. (2007).
Gymnogeophagus sp.	48	50	2 M/SM + 46 ST/A	Paraná river, Misiones, Argentina	Roncati et al. (2007).
Geophagus brasiliensis	48	54	6 M/SM + 42 ST/A	São Mateus do Sul, PR, Brazil	Kantek et al. (2007).
Crenicichla inpa	48	54	6 M/SM + 42 ST/A	Br 174, km 14, AM, Brazil	Benzaquem et al. (2008).
Crenicichla cf. *johanna*	48	56	8 M/SM + 40 ST/A	Catalão, AM, Brazil	Benzaquem et al. (2008).
Crenicichla lugubris	48	56	8 M/SM + 40 ST/A	Catalão, AM, Brazil	Benzaquem et al. (2008).
Crenicichla lepidota	48	56	8 M/SM + 40 ST/A	São Gonçalo-Mangueira Basin, RS, Brazil	Perazzo et al. (2011).
Crenicichla trigata	48	54	6 M/SM + 42 ST/A	Das Mortes river, MT, Brazil	Valente et al. (2012).
Satanoperca jurupari	48	52	4 M/SM + 44 ST/A	Das Mortes river, MT, Brazil	Valente et al. (2012).
Biotodoma cupido	48	52	4 M/SM + 44 ST/A	Das Mortes river, MT, Brazil	Valente et al. (2012).
Geophagus cf. *proximus*	48	60	12 M/SM + 36 ST/A	Água Preta Lake, PA, Brazil	Present paper

2n = diploid number; FN = Fundamental Number; KF = Karyotypic Formulae; M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric. Abbreviations refer to Brazilian states: AM: Amazonas; PR: Paraná; MT: Mato Grosso; MS: Mato Grosso do Sul; RS: Rio Grande do Sul; PA: Pará.

the ratio between the chromosomal arms necessary to confirm the class they belong to, in accordance with the method described by Levan et al. (1964).

Results and discussion

The analysis of 120 metaphases (30 of each individual) revealed a karyotype of 2n = 48 and FN = 60 for all specimens (Figure 1), consisting of two metacentric pairs, four submetacentric pairs and 18 subtelo-acrocentric pairs. Even though male and female specimens were analyzed, no chromosomal differences were found between the sexes. The analysis using Ag-NOR technique showed only a single mark, interstitially located in the short arms of the second M pair, in all metaphases (Figure 1, box). The NOR-band positive marks are C-band negative and we observed some heteromorphism between the marked NORs.

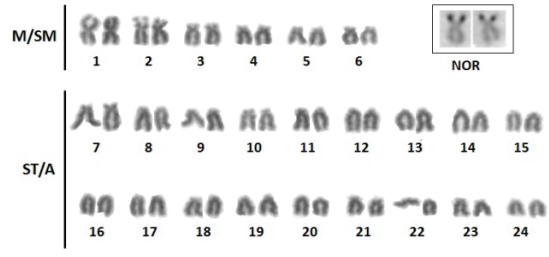

Figure 1. Conventional Giemsa-stained karyotype of *Geophagus* cf. *proximus* from Água Preta Lake - Belém, Pará State, Brazil. The silver nitrate-nucleolus organizer region (Ag-NOR) chromosome pair is evident.

The analysis of the C-banding pattern revealed large blocks of constitutive heterochromatin located on the pericentromeric regions of all chromosomes (Figure 2), and the first pair presented a completely heterochromatic long arm.

The Cichlidae family is considered a group with conserved chromosomal macrostructure, where most of the analyzed species have a diploid number of 48 chromosomes (FELDBERG et al., 2003). This characteristic has been proposed by some authors as a conserved trait for this family, suggesting that the ancestral karyotype consisted of 48 acrocentric chromosomes (THOMPSON, 1979; MARTINS et al., 1995; FELDBERG et al., 2003; PERAZZO et al., 2011).

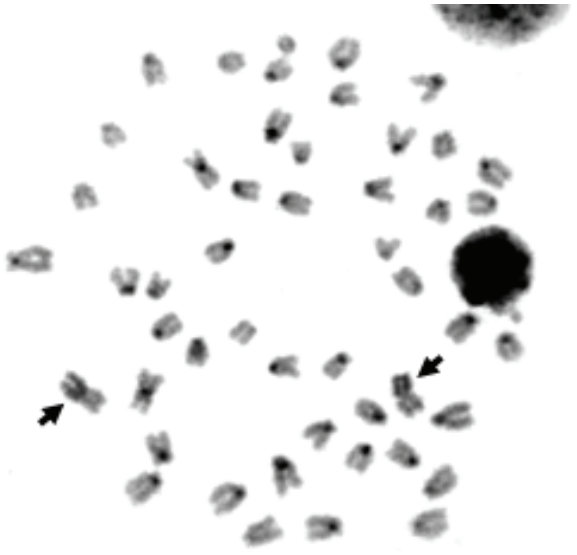

Figure 2. C-banded metaphase of *Geophagus* cf. *proximus* showing a large pericentromeric band in all chromosomes (dark regions). Arrows indicate chromosomes of the first pair with long arm completely stained.

Based on cytogenetic data of 41 Neotropical cichlid species, Thompson (1979) inferred that the karyotype in this fish group has been enough conservative, with a diploid number (2n) about 48 and FN ranging from 48 to 70. This inference has been confirmed by many recent studies (LOUREIRO, 1999; MARESCALCHI, 2005; MIZOGUCHI et al., 2007; BENZAQUEM et al., 2008; VALENTE et al., 2012).

A karyotype composed by 2n = 48 chromosomes with occurrence of metacentric and submetacentric

chromosomes, as observed in *Geophagus* cf. *proximus*, represents a derived condition for the Cichlidae family, whose basal karyotype would be composed by 2n = 48 acrocentric chromosomes. This derived condition has been also cited in other recent researches, like Perazzo et al. (2011) with *Crenicichla lepidota* and *Australoheros facetus*.

Data regarding chromosomal rearrangements in Neotropical cichlids have been obtained mainly through conventional cytogenetic studies (Table 1). Thompson (1979), Vervoort (1980) and Uribe-Alcocer and Arreguín-Espinosa (1989) indicated that several Cichlidae species, both phylogenetically close and distant, show small differences in karyotype. The existing diversity may be caused by both gains or losses of small chromosomal fragments, non-reciprocal translocations, pericentric inversions, duplications or uneven crossing-over (URIBE-ALCOCER et al. 1992), as well as non-Robertsonian translocations (PERAZZO et al., 2011). Although the cichlid cytogenetics suggests that the ancestral karyotype (2n = 48 ST/A) could have undergone major changes (pericentric inversions, fusions, fissions and chromosomal translocations) in the macro-structure of the South American species (FELDBERG et al., 2003, POLETTO et al., 2010). The Ag-NOR technique in *Geophagus* cf. *proximus* showed the presence of single marks, subterminally located in the short arm of the second pair in all specimens (Figure 1, box). We also found some heteromorphism between the marked NOR. NOR had a negative C band, as observed by Mizoguchi et al. (2007) in *Crenicichla*.

Loureiro (1999) obtained the same pattern (NORs in only one chromosome pair) for two *Crenicichla* species, with NORs located on the short arm of the first metacentric chromosome pair, and for *Geophagus brasiliensis*, on the short arm of the larger pair of the ST-A group. However, in *Cichlasoma paranaense,* two patterns were observed. The first pattern, found in individuals collected from the Guaravera region (Londrina, Paraná State, Brazil), exhibited more than one homologous chromosome pair by $AgNO_3$ staining, suggesting multiple NORs; the second pattern, found in individuals collected in other points of the Tibagi river basin (Paraná State, Brazil) exhibited a single nucleolar chromosome pair, staining the short arm of the second pair. Differently from our results, this author found a significant heteromorphism in the NOR staining in all Cichlidae species analyzed, even in metaphases from the same individual.

Almeida-Toledo and Foresti (1985) studied the NOR-banding pattern in 48 fish species of the Anostomidae, Sternopygidae, Parodontidae, Characidae and Cichlidae families, and found only one mark in all species. This is similar to our results, which is predominant among fish, and since those authors also had inferred that the karyotype with one unique NOR pair may be considered ancestral of those whose NORs are located in multiple chromosomes. The predominance of single marks has been confirmed in more recent studies (ANDREATA et al., 2006; BRINN et al., 2004; BENZAQUEM et al., 2008; MILHOMEM et al., 2008; MIZOGUCHI et al., 2007; MORAES et al., 2007; NIRCHIO et al., 2003; PORTO-FOREST et al., 2008; RONCATI et al., 2007; SHIMABUKURO-DIAS et al., 2004).

According to Feldberg et al. (2003), the general trend in the Cichlidae family is a single NOR system, with only one pair of NORs located on the largest chromosome of the complement. This chromosomal character seems to be plesiomorphic for ciclids.

Usually, C-banding pattern observed in Cichlidae species is restricted, involving chromosome portions in centromeric and/or pericentromeric positions. To some extent *Geophagus* cf. *proximus* followed this tendency, since it presents positive C-bands restricted to the pericentromeric region of all chromosomes (Figure 2). This is the pattern found for all the analysed cichlid species both from the New World and Old World (FELDBERG et al., 2003).

Moreover, the largest chromosome pair presented its long arm completely heterochromatic. The detection of this apomorphy can be correlated to ecological peculiarities of this species, whose studied population is relatively isolated in the lakes inside the Parque Ambiental de Belém. Reports on heterochromatin variation in fish species (MANTOVANI et al., 2004) or other vertebrate groups (PATHAK et al., 1973) are usually associated to restricted and small populations, susceptible to higher rates in chromosomal evolution (KING, 1987; MOREIRA-FILHO; BERTOLLO, 1991).

Benzaquem et al. (2008) investigated karyotypes of six cichlid species of the genus *Crenicichla.* The heterochromatin patterns of the species analyzed were very similar; however, some species exhibited at least one species–specific block. These unique heterochromatic blocks are chromosomal markers and the authors suggested that they can also be used in further comparisons among several *Crenicichla* species.

Similarly, our observation that the first chromosomic pair (metacentric) had its long arm completely stained shows the possibility of using C-banding at least as a population marker.

Conclusion

The conventional cytogenetic analysis with C-banding and NOR-staining revealed that *Geophagus* cf. *proximus* shows the number of chromosomes and C-banding pattern more common among cichlids; fundamental number is within the range characteristic of the family, also the standard NOR had been found in other species. The novelty was the chromosomes of the first pair with long arm completely heterochromatic, which may be an important cytogenetic marker. Only after further studies, with confirmation of the species, including different populations, may be set if this marker is species-specific.

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