PL-ISSN 0015-5497 (print), ISSN 1734-9168 (online)
Folia biologica (Kraków), vol. **58** (2010), No 1-2

doi:10.3409/fb58_1-2.29-34

Cytogenetic Characterization of *Geophagus brasiliensis* and Two Species of *Gymnogeophagus* (Cichlidae: Geophaginae) from Guaíba Lake, RS, Brazil

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Accepted September 15, 2009

BETTIN PIRES L., GIULIANO-CAETANO L., DIAS A. L. 2010. Cytogenetic characterization of *Geophagus brasiliensis* and two species of *Gymnogeophagus* (Cichlidae: Geophaginae) from Guaíba Lake, RS, Brazil. Folia biol. (Kraków) **58**: 29-34.

The karyotypes of three species of fish of the Cichlidae family from the Forqueta river and several locations in Guaíba lake/RS (Brazil) were analyzed. All species presented 2n=48, while *Gymnogeophagus gymnogenys* showed two karyotypic formulae: 4m+44st-a with FN=52 and 6m+42st-a with FN=54. *Gymnogeophagus labiatus* presented 4m+4sm+40st-a and FN=56 and *Geophagus brasiliensis* 4sm+44st-a and FN=52. Simple NORs were found in all species with the exception of a population of *G. gymnogenys* from Saco da Alemoa/Barra do Ribeiro. CMA_3 staining revealed NOR sites, while DAPI staining was negative and heterochromatin was limited to pericentromeric regions and associated to NORs, except in *G. labiatus*. The data show a conserved pattern in *Geophagus brasiliensis* and karyotype variation in the species of *Gymnogeophagus*.

Key words: Chromosome banding, diploid number conservation, karyotype, Perciformes.

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Among the Perciformes, the Cichlidae is one of the largest families of vertebrates with 1300 described species and up to an estimated 1870 species. Only the fish families Gobiidae and Cyprinidae, with 1875 and 2010 species, respectively, compete for the position of the largest family (KULLANDER 1998).

The distribution of species of cichlid fishes is wide and includes Africa, South America, Central America and parts of Asia and North America, in a diversity of habitats (MOYLE & JUNIOR CECH 2000).

The South American cichlids comprise about 50 genera and 450 species and were organized by KULLANDER (1998) into eight subfamilies: Etroplinae, Pseudocrenilabrinae, Retroculinae, Cichlinae, Heterochromidinae, Astronotinae, Geophaginae and Cichlasomatinae.

The subfamily Geophaginae is represented by 18 genera, but little is known of the cytogenetics of this group. Information is available for only seven genera: *Acarichthys*, *Apistogramma*, *Dicrossus*, *Geophagus*, *Guianacara*, *Gymnogeophagus* and *Satanoperca*, in which *Geophagus brasiliensis* (QUOY & GAIMARD 1824) is cytogenetically the best known. Most of the data obtained for this subfamily concern only the description of the diploid number, karyotype formula and the location of NORs.

In this study we cytogenetically analyse three species from two geophaginae genera, *Gymnogeophagus* and *Geophagus*, from Guaíba lake and Forqueta river, RS, Brazil, through different chromosome banding techniques.

Material and Methods

Were analysed three species of the subfamily Geophaginae: 15 specimens (8 males, 5 females and 2 individuals of undetermined sex) of *Gymnogeophagus gymnogenys* (HENSEL 1870), 13 specimens (5 males, 5 females and 3 individuals of undetermined sex) of *G. labiatus* (HENSEL 1870) and 10 individuals (5 males and 5 females) of *Geophagus brasiliensis*, collected in the Forqueta river and three different localities in Guaíba lake, RS: Barra do Ribeiro, Gasômetro and Saco da Alemoa (Table I). Voucher specimens are catalogued in the fish collection of Museu de Zoologia da Universidade Estadual de Londrina under catalog numbers: MZUEL 4050 – *Gymnogeophagus gymnogenys*;

Table 1

Summary of the results for *Gymnogeophagus* and *Geophagus* in this work (2n = diploid number, FN = fundamental number, SC = secondary constriction, NORs = nucleolar organizer regions, HC = heterochromatin and CMA_3 = chromomycin A_3)

Species	Locality	2n	Karyotype formula	FN	SC	NORs	HC	CMA_3
Gymnogeophagus gymnogenys	Saco da Alemoa and Barra do Ribeiro (RS)	48	4 m + 44 st-a	52	Pair 3 (i)*	Multiple: Pair 3 (i)* Pair 9 (t)	C	Pair 3 (i)* Pair 9 (t)
	Gasômetro (RS)	48	6 m + 42 st-a	54	Pair 20 (t)*	Simple: Pair 20 (t)*	C	Pair 20 (t)*
Gymnogeophagus labiatus	Saco da Alemoa and Forqueta river (RS)	48	4 m + 4 sm + 40 st-a	56	Pair 1 (i)	Simple: Pair 1 (i)	C	Pair 1 (i)
Geophagus brasiliensis	Saco da Alemoa and Gasômetro (RS)	48	4 sm + 44 st-a	52	Pair 6 (t)*	Simple: Pair 6 (t)*	C	Pair 6 (t)*

Legend: t=terminal; i=intersticial; C=pericentromeric; *=heteromorphism

MZUEL 4901 – *Gymnogeophagus labiatus* and MZUEL 4842 – *Geophagus brasiliensis*.

Mitotic chromosomes were obtained from kidney according to the conventional technique of BERTOLLO *et al*. (1978). Chromosome morphology was determined on the basis of arm ratio in metacentric (m), submetacentric (sm) and subtelocentric-acrocentric (st-a) chromosomes, according to LEVAN *et al*. (1964), with modifications. Chromosomes classified as metacentric and submetacentric were considered as biarmed, and subtelocentric-acrocentric as uniarmed, to determine the fundamental number (FN). Nucleolar organizer regions (NORs) were identified by silver nitrate staining according to HOWELL and BLACK (1980). Heterochromatin was located by the C-banding technique using barium hydroxide according to SUMNER (1972). Fluorochrome staining with the GC- specific chromomycin-A_3 (CMA_3) and AT-specific diamidino-2-phenylindole (DAPI) was carried out according to SCHWEIZER (1980).

Results

The results for the three species revealed a diploid number (2n) equal to 48 chromosomes; however, differences in karyotype formula were observed. *Gymnogeophagus gymnogenys* presented 4m + 44st-a and a fundamental number (FN) equal to 52 for the population of Barra do Ribeiro and Saco da Alemoa (Fig. 1a), and 6m + 42st-a and FN = 54 for the population of Gasômetro (Fig. 1b); *Gymnogeophagus labiatus* presented 4m + 4sm + 40st-a and FN = 56 (Fig. 2a) and *Geophagus brasiliensis* 4sm + 44st-a and FN = 52 (Fig. 3a) (Table 1).

A secondary constriction in *G. gymnogenys* is located in an interstitial position on the long arm of pair 3 (st-a) for the population from Saco da Alemoa/Barra do Ribeiro, and in the terminal region on the short arm of pair 20 (st-a) for Gasômetro, showing size heteromorphism among the homologues (Fig. 1a & 1b, respectively). In *G. labiatus* the secondary constriction is located interstitially on the short arm of the largest pair of the complement (Fig. 2a) and in *Geophagus brasiliensis* in the terminal position of pair 6 (st-a), visible in some analyzed samples, showing heteromorphism between homologous chromosomes (Fig. 3a).

Silver nitrate staining of the chromosomes showed simple NORs in *Gymnogeophagus labiatus*, *G. gymnogenys* (Gasômetro population) and *Geophagus brasiliensis.* One pair of metacentric chromosomes with interstitial AgNORs on their short arms was observed in the first species (Fig. 2b), and terminal AgNORs on the short arms of one subtelo-acrocentric pair, with size heteromorphism, were observed in the last two species, all coincident with secondary constrictions (Fig. 1d & 3b, respectively).

In the samples of *G. gymnogenys* from Saco da Alemoa/Barra do Ribeiro, silver nitrate staining revealed multiple NORs, with 2 to 4 nucleolar chromosomes and markings in the terminal region of the short arm in a pair of subtelo-acrocentric chromosomes (pair 9) and in the interstitial region of the long arm of pair 3 (st-a). The latter pair was coincident to secondary constriction which showed size heteromorphism between the homologues of the region (Fig. 1c, Table I).

Staining with the fluorochrome chromomycin A_3 revealed fluorescent sites corresponding to the

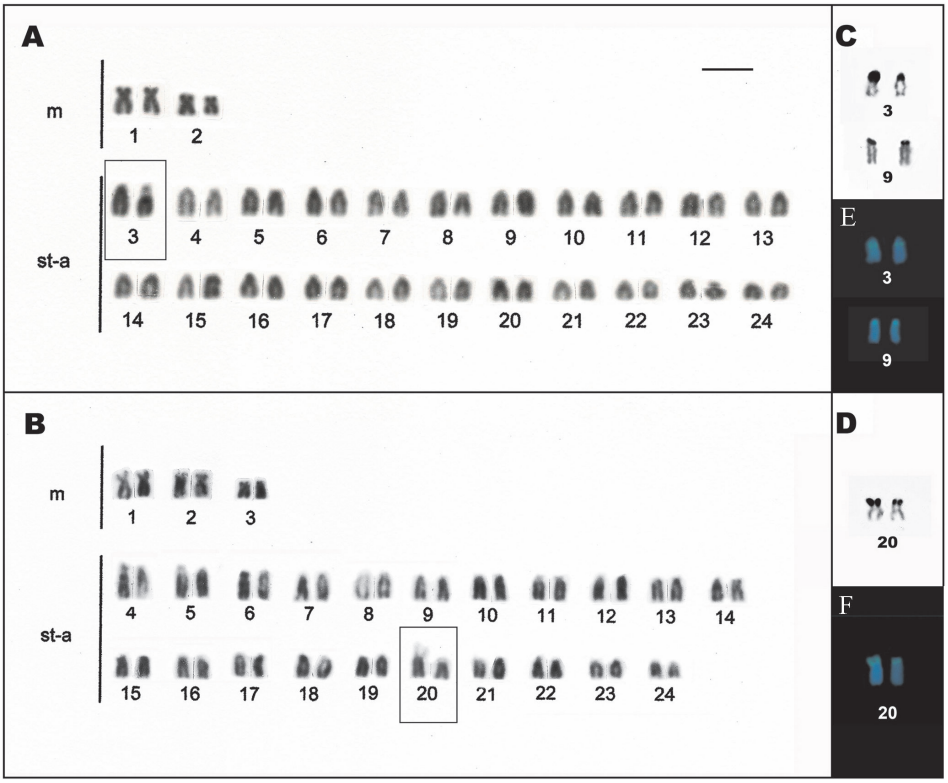

Fig. 1. Karyotypes of *Gymnogeophagus gymnogenys*, of the Saco da Alemoa/Barra do Ribeiro (a) and Gasômetro (b), with AgNORs (c and d, respectively) and CMA_3 pairs (e and f, respectively). Bar = 10 μm.

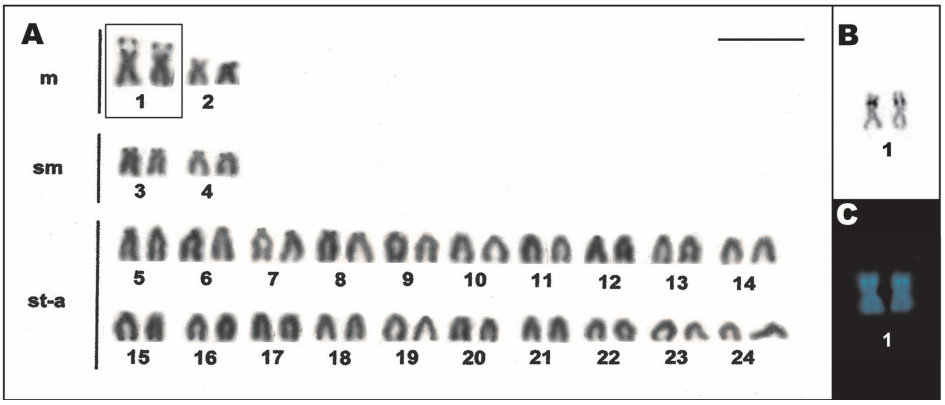

Fig. 2. Karyotype and AgNORs/CMA_3 pairs of *Gymnogeophagus labiatus* (a, b and c, respectively). Bar = 10 μm.

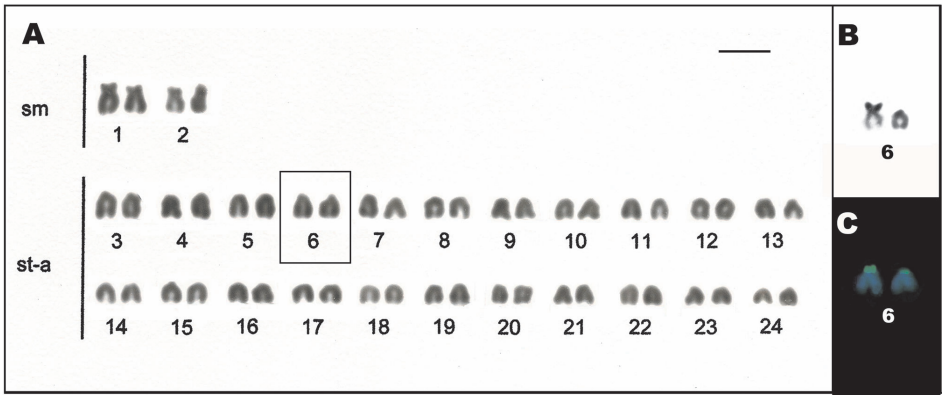

Fig. 3. Karyotype and AgNORs/CMA_3 pairs of *Geophagus brasiliensis* (a, b and c, respectively). Bar = 10 μm.

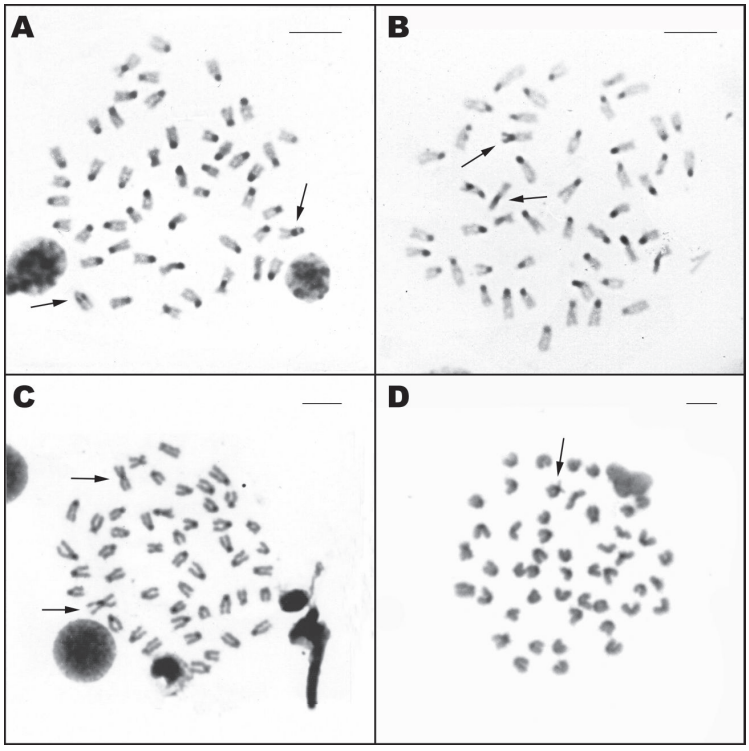

Fig. 4. Somatic metaphases with C banding: *G. gymnogenys* from Saco da Alemoa/Barra do Ribeiro (a) and Gasômetro (b), *G. labiatus* (c) and *Geophagus brasiliensis* (d). The arrows indicate the chromosome with the secondary constrictions. Bar = 10 μm.

AgNORs in all species. A fluorescent signal was not observed for DAPI, whereas the secondary constriction was less evident in some species (Fig. 1e and f, Fig. 2c, Fig. 3c).

C banding revealed heterochromatin distributed mainly in the pericentromeric regions of several chromosomes and heterochromatin blocks associated with NORs, except in *G. labiatus* (Fig. 4). The same size heteromorphism observed for Giemsa and silver staining in *G. gymnogenys* and *G. brasiliensis* was also found for C banding.

Discussion

The species studied here showed a diploid number of 48 chromosomes, coincident to the number described for other species of *Geophagus* and *Gymnogeophagus* (FELDBERG & BERTOLLO 1985; PEIXOTO & ERDTMANN unpublished data; BRUM *et al*. 1998; VICARI *et al*. 2006; PIRES *et al*. 2008) and for other genera of the subfamily Geophaginae (FELDBERG *et al*. 2003). However, different chromosome numbers were reported in *Apistogramma borellii* (2n = 38), in *Apistogramma agassizii*, *A. ortmanni*, *A. steindachneri* and *Dicrossus filamentosus* (2n = 46) by THOMPSON (1979), showing some karyotypical variation in this subfamily.

FELDBERG *et al.* (2003) suggested that the diploid number of 48 chromosomes is the basal condition in Neotropical cichlids, since it is found in most species, and is composed usually of the st-a chromosome type, resulting in a small fundamental number.

Although the diploid number is conservative in the different species of *Gymnogeophagus* and *Geophagus brasiliensis* studied so far, changes in karyotype formula were found between species and within the same species (FELDBERG *et al.* 2003), as observed here in *G. gymnogenys* and *G. labiatus*.

PEIXOTO & ERDTMANN (personal communication) have studied the same species of *Gymnogeophagus* from two different river drainages: from the Jacuí river, a tributary of Guaíba lake and belonging to the same drainage as the Forqueta river (Patos lagoon system); and from the Tramandaí river drainage, an isolated coastal drainage to the north of the Patos lagoon system. They found a karyotype formula of 4m-sm + 44st-a and NF = 52. This constitution was the same as that for the *G. gymnogenys* population from Saco da Alemoa/Barra do Ribeiro (Table 1). Karyotype variation found in the two species of *Gymnogeophagus* is probably due to chromosomal rearrangements of the pericentric inversion type.

The genera *Gymnogeophagus* and *Geophagus* belong to the geophaginae cichlid group which is characterized by a series of synapomorphies including an expanded epibranchial lobe (WIM-

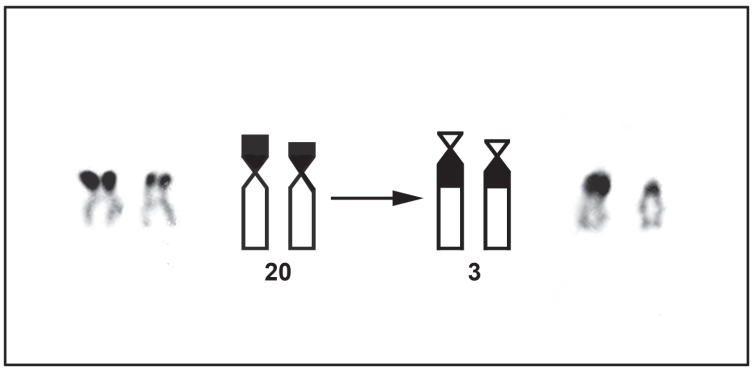

Fig. 5. Scheme showing the possible occurrence of a pericentric inversion on pair 20 in *G. gymnogenys* from Gasômetro, with the NOR in terminal position, originating from pair 3 in the population Saco da Alemoa/Barra do Ribeiro, with the NOR in interstitial position. Both pairs of chromosomes present the same size heteromorphism in this region.

BERGER *et al.* 1998). Although this is a monophyletic group, each species studied here has some specific cytogenetic pattern.

Geophagus brasiliensis has been examined by several authors and, despite the constant 2n = 48, variation has been described in the karyotype formula (FELDBERG & BERTOLLO 1985; MARTINS *et al*. 1995; BRUM *et al*. 1998; VICARI *et al.* 2006). The karyotypical constitution of 4sm + 44st-a, found here is similar to that described by THOMPSON (1979) and PIRES *et al*. (2008).

KULLANDER (1998) and FARIAS *et al.* (2000) based on morphological and molecular characters, respectively, proposed that "*Geophagus brasiliensis*" seems to constitute a complex of species. Data in the cytogenetic review by FELDBERG *et al.* (2003), added to the present study, corroborate this hypothesis since variation in 2 to 8 chromosomes of the m-sm type between populations has been observed, indicating the existence of cryptic species in this group (BRUM *et al.* 1998) or an initial process of speciation.

A simple NOR pattern was detected in *Gymnogeophagus labiatus* and *Geophagus brasiliensis*, following the general pattern in the family Cichlidae, in which the AgNORs are located in the larger chromosome pairs of the complement, either in the m-sm or st-a group, corresponding to a probable plesiomorphic character for this group of fish (FELDBERG *et al.* 2003).

PEIXOTO & ERDTMANN (personal communication) observed the same AgNOR pair of the m type in *G. labiatus* from the Jacuí river and Tramandaí river drainages, as reported in this study. Different populations of *G. brasiliensis* (VICARI *et al*. 2006; PIRES *et al*. 2008; among others) also showed simple NORs in st-a chromosomes. However, mosaicism of NORs has already been described in *G. brasiliensis* from Rodrigo de Freitas lagoon, RJ, which represents the only reference of multiple NORs for this species (BRUM *et al*. 1998).

We detected variation in NORs within *G. gymnogenys*. The population of Gasômetro presented simple NORs in pair 20, considered an ancestral condition for cichlids, and the population of Saco da Alemoa/Barra do Ribeiro had multiple NORs in pairs 3 and 9, as summarized in Table I, indicating that the latter populations have more derivative traits than the population of Gasômetro. Therefore in Figure 5 we present the hypothesis that pair 20 of *G. gymnogenys* from Gasômetro with a terminal NOR may have originated from pair 3 from Saco da Alemoa/Barra do Ribeiro with interstitial marks, through a pericentric inversion, since both pairs have NOR heteromorphism of the same size.

The data obtained with the fluorochrome chromomycin A_3 indicated that the NORs in all species are rich in GC pairs and therefore CMA_3 positive, which is the general pattern found in most cichlids. We did not detect a DAPI signal, indicating that the chromosomes of these species lack regions rich in AT pairs. The data from CMA_3 and DAPI staining reported in the literature concern only *G. brasiliensis* (VICARI *et al.* 2006; PIRES *et al*. 2008), the results of which are the same as those obtained herein.

The heterochromatin pattern was similar in all species analyzed, being preferentially distributed in the pericentromeric regions of the majority of chromosomes, with heterochromatic blocks associated to NORs, except in *G. labiatus* in which these regions were negative after C banding. This may be considered a characteristic of this particular species, since the presence of heterochromatin associated with the ribosomal cistrons has been found in several species of this family, such as *Satanoperca papaterra* (MARTINS *et al*. 1995), *Crenicichla niederleinii* (LOUREIRO *et al*. 2000), *Cichlasoma facetum* [=*Australoheros facetus*] (VICARI *et al*. 2006), and *Geophagus brasiliensis* (PIRES *et al*. 2008), among others.

The heterochromatic pattern found for *G. brasiliensis* showed similarities to other analysed

populations of this species (FELDBERG & BERTOLLO 1985; MARTINS *et al*. 1995; BRUM *et al*. 1998; VICARI *et al*. 2006; PIRES *et al*. 2008), except the population from the Jaguariaíva river studied by VICARI *et al*. (2006), which showed some chromosomal pairs with interstitial markings that differ from the pattern in *G. brasiliensis*.

The data obtained here confirm that *Geophagus brasiliensis* has conserved cytogenetic characteristics in relation to other populations of the studied species, and that the differences mainly concern karyotype formulas. These may be the result of chromosomal rearrangements, such as pericentric inversions, indicating a possible initial process of speciation.

However, in the genus *Gymnogeophagus* the karyotype, location of NORs and distribution of heterochromatin is variable in the two species studied here, as well as *G. gymnogenys* from different locations, which may indicate a more divergent karyotypical evolution in this genus.

Acknowledgements

The authors are grateful to CAPES, CNPq and Fundação Araucária for financial support and to M.Sc. Ana Paula S. DUFECH for facilities in fish capture and Dr. Luiz R. MALABARBA for the identification of the studied species and critical review of the manuscript.

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