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Evidence of the color pattern variation in populations of *Gymnotus pantanal* (Gymnotiformes) from three streams in the upper Paraná River basin, Brazil

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Color pattern is an important character in the systematics and alpha-taxonomy of electric fishes of the genus *Gymnotus*. This paper presents evidence of color variation in populations of *G. pantanal* found in the streams Jacutinga and Pinheirinho, in the upper Paraná River basin, southern Brazil. Color variations were corroborated for morphological and cytogenetic data. Our results show the importance of integrating morphologic and cytogenetic data in the taxonomy of the *Gymnotus* species.

O padrão de colorido é um caráter muito importante na sistemática e alfa taxonomia de espécies do gênero *Gymnotus*. O objetivo deste trabalho foi apresentar evidências de variação no padrão de colorido em populações locais de *Gymnotus pantanal* encontrados nos córregos Jacutinga e Pinheirinho, bacia do alto rio Paraná, sul do Brasil. A variação no padrão de colorido foi corroborada por dados morfológicos e citogenéticos. Nossos resultados demonstram a importância da integração de dados morfológicos e citogenéticos na taxonomia de espécies de *Gymnotus*.

Key words: Gymnotidae, *Gymnotus pantherinus* species-group, La Plata River basin, Karyotype.

Introduction

The genus *Gymnotus* Linnaeus comprises 35 valid species of electric fishes, including aggressive and nocturnal gymnotiform eels of shallow freshwaters distributed from southern Mexico to Argentina (Cognato *et al*., 2007; Maxime & Albert, 2009; Richer-de-Forges *et al*., 2009). *Gymnotus pantanal* was described by Fernandes *et al*. (2005) in the Paraná-Paraguay system, in Brazil and Paraguay, and in the River Chapare-Mamoré, in Bolivia. Fernandes *et al*. (2005) used morphological, cytogenetic, and molecular data to describe that species; in their diagnosis color pattern was the main morphological trait: '*Gymnotus pantanal* differs from other members of the *G. pantherinus* Steindachner species-group (except *G. anguillaris* Hoedeman) in possessing a color pattern composed of thin obliquely-oriented pale pigment bands (about one third the width of the dark bands) with wavy margins restricted to the ventral portion of the body (rarely extending above the lateral line) on the anterior half of the body'. Later, Graça & Pavanelli (2007) and Margarido *et al*. (2007) reported this species for the upper Paraná River basin.

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Despite the high diversity of this genus, only eight species of *Gymnotus* have been cytogenetically analyzed: *G. paraguensis* Albert & Crampton, 2003, *G. pantherinus* (Steindachner, 1908), *G. inaequilabiatus* (Valenciennes, 1839) *G. sylvius* Albert & Fernandes-Matioli, 1999 (Fernandes-Matioli *et al*., 1998), *G. pantanal* Fernandes, Albert, Daniel-Silva, Lopes, Crampton & Almeida-Toledo, 2005 (Fernandes *et al*., 2005; Silva & Margarido, 2005), *G. mamiraua* Albert & Crampton, 2001 (Milhomem *et al*., 2007), *Gymnotus* sp. (Lacerda & Maistro, 2007) and *G*. cf. *carapo* Linnaeus, 1758 (Milhomem *et al*., 2008). Diploid numbers vary from 39-40 (*G. pantanal*) to 54 (*G. paraguensis*), and differences in karyotypic macrostructure were observed among the analyzed species. However, *G. pantanal* is the only species of the genus with a multiple sex chromosome system (Silva & Margarido, 2005).

In samples collected recently in two streams in the urban area of Toledo, upper Paraná River basin, southern Brazil, some specimens of *Gymnotus* exhibited a different color pattern. Considering the importance of color in the taxonomy of *Gymnotus*, we analyzed those specimens based on morphological and cytogenetical data with the main objective to investigate if the specimens with different color patterns belongs to the species *G. pantanal*.

Material and Methods

Study area

This study was carried out in three first-order streams (Strahler, 1957; Fig. 1) located within the urban perimeter of Toledo, State of Paraná, southern Brazil, within the sub-basin Paraná III. This sub-basin is composed of several micro-basins with peculiar characteristics, and, among those, the micro-basin of the River São Francisco Verdadeiro stands out. This micro-basin is formed by several rivers and streams, and due to human activities, it became a highly-impacted area (Gubiani *et al*., 2010). As a result, the streams flowing in the city show different pollution levels. The micro-basin of the river São Francisco Verdadeiro flows through the cities of Cascavel, Toledo, Ouro Verde do Oeste, São José das

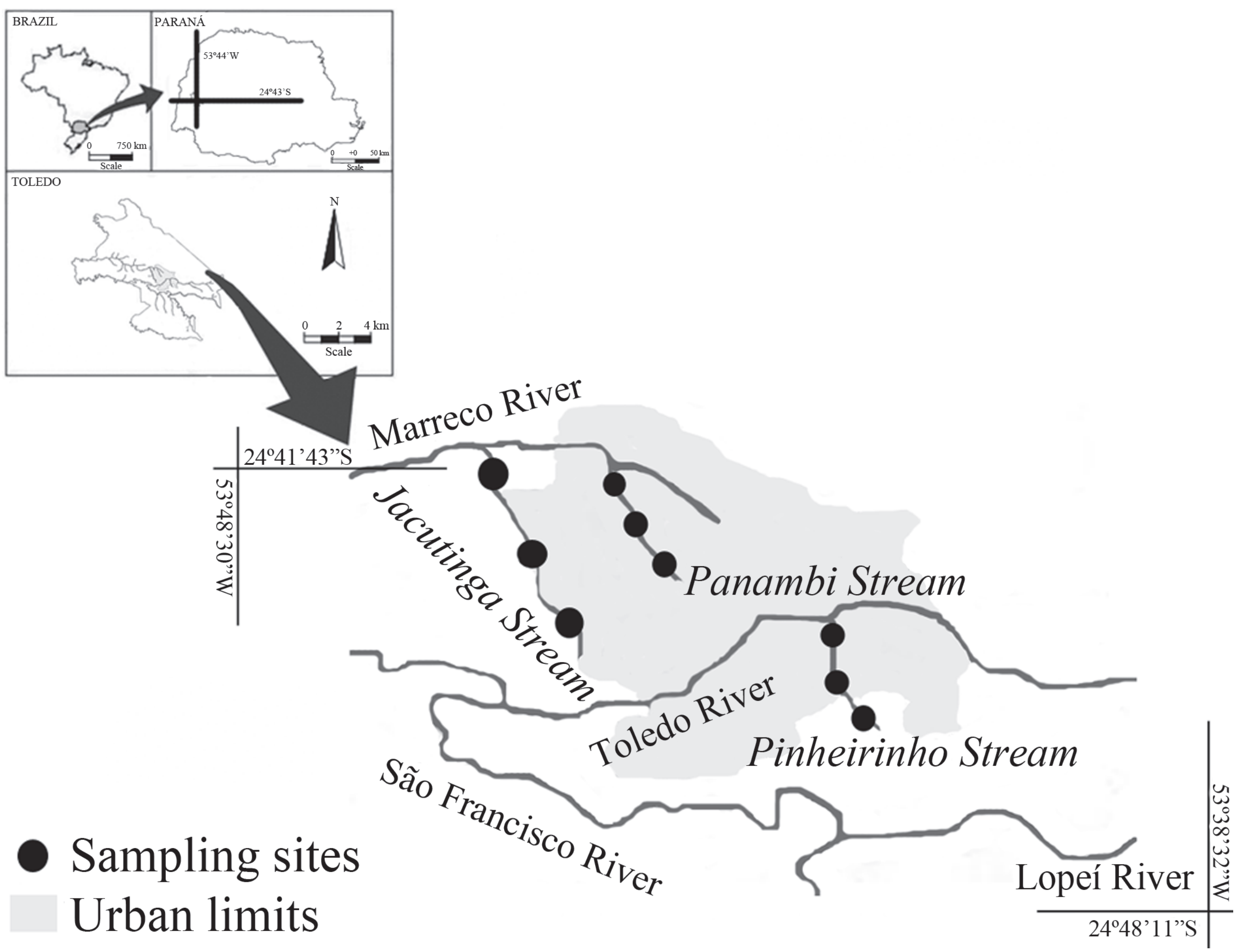

Fig. 1. Partial map of São Francisco Verdadeiro River, Toledo, State of Paraná, showing the location of sites studied.

Table 1. Morphometric and meristics data of *G. pantanal*. The data from *G. pantanal sensu stricto* follows Fernandes *et al*. (2005). Total length and head length expressed in mm. Anal-fin length, body depth, and body width as percentage total length. Other measurements are expressed as percentage head length. *Gymnotus pantanal* typical color pattern n = 25 and atypical color pattern n = 16. M is the mean and Range is the minimum and maximum value.

	Gymnotus pantanal					
Characters	*Sensu stricto*		Typical color pattern		Atypical color pattern	
Morphometrics	Range	M	Range	M	Range	M
Total length (mm)	84.0-251.0	-------	139.4-260.3	-------	113.4-248.0	-----
Head length (mm)	9.7-21.5	-------	14.0-28.9	-------	13.2-23.8	-----
Head length	8.6-10.1	9.1	9.0-11.0	9.4	9.1-11.2	9.5
Preorbital length	34.0-37.0	35.0	28.4-40.6	32.6	28.0-39.0	32.5
Postorbital length	59.0-62.0	60.0	54.5-60.0	58.8	54.4-64.4	59.4
Mouth width	44.0-48.0	46.0	38.7-47.9	45.9	43.0-50.3	46.2
Interorbital length	37.0-41.0	39.0	33.9-42.5	38.2	33.1-40.5	36.6
Head depth	66.0-74.0	70.0	63.8-73.4	69.0	57.1-70.6	66.6
Branchial opening	38.0-43.0	40.0	37.8-42.9	39.9	37.5-41.9	39.4
Body depth	8.0-9.5	8.8	8.2-10.7	9.0	6.8-11.0	8.9
Body width	6.0-7.2	6.5	6.0-7.0	6.2	5.5-7.2	6.3
Body width/body depth	0.7-0.8	0.7	0.6-0.8	0.7	0.6-0.9	0.8
Pectoral fin length	51.0-56.0	53.0	47.0-54.1	52.3	47.1-53.7	49.9
Anal-fin length	78.0-81.0	80.0	76.0-83.5	78.4	78.0-82.1	79.0
		Meristics				
Color bands	7-25	13	11-24	16	-----------	-----
Pectoral fin rays	16-18	17	16-18	17	16-18	17
Scales above lateral line	7-8	8	7-8	8	7-8	8
Scales to first ramus	47-58	54	49-56	53	51-57	54
Scales to last lateral-line pore	102-114	108	100-117	109	100-114	109
Anal-fin rays	235-280	258	239-274	260	240-276	262

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Three streams were selected in Toledo: Panambi, Pinheirinho, and Jacutinga. The headwaters of the stream Panambi are located downtown; its margins are totally occupied by residences, and it receives domestic and industrial sewage. The headwaters of the stream Pinheirinho are located outside the city and are affected by agriculture; in its medium portion the stream is also impacted by residences. The stream Jacutinga is less affected by residences; though it is affected by aquaculture and agriculture (see Gubiani *et al*., 2010).

Fish sampling

Fish were sampled bimonthly from October 2007 to February 2009 in the selected streams at three different zones (headwater, middle and mouth) (Fig. 1). The length of the sampling transect in each site was 40 m, which is slightly longer than recommended (35 times the stream's width, Simonson & Lyons 1995). We used electrofishing, an efficient method for collecting small species (Severi *et al*., 1995), in lotic environments (Mazzoni *et al*., 2000). The electrofishing equipment was powered by a portable generator (HONDA, 2.5 kW, 220 V, 3-4 A) connected to a DC transformer and two electrified net rings (anode and cathode). Output voltage varied from 400 to 600 V. Each transect was sampled three times from downstream to upstream by four people in a constant fishing effort of 30 min, following Esteves & Lobón-Cerviá (2001). Both extremities of the sampled transect were blocked by a net (0.5 cm mesh) to prevent fish from getting in and out of the sampling site. Institutional acronyms are described on the website: http://research.calacademy.org/research/ichthyology/catalog/abtabr.html. Voucher specimens [NUP 9311 (5) and NUP 9312 (11)] were deposited in the fish collection of Nupélia (Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura), at Maringá State University, Brazil (http://www.nupelia.uem.br/colecao).

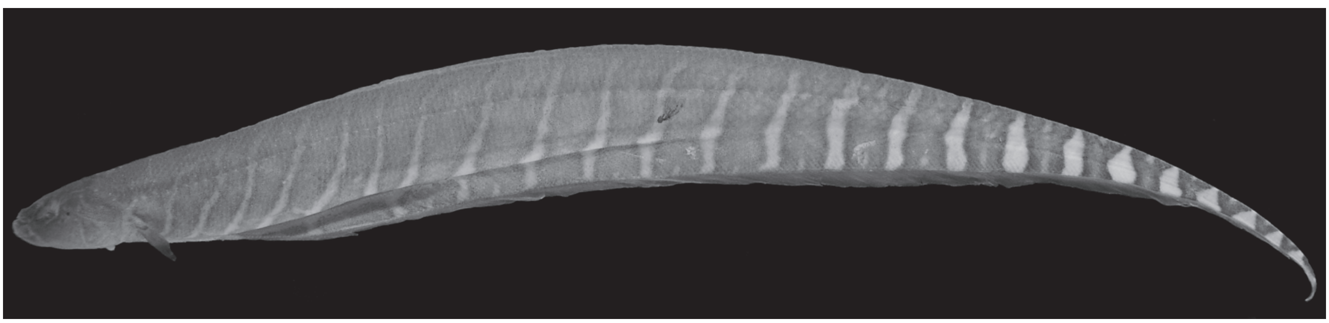

Fig. 2. *Gymnotus pantanal*, holotype, 196.0 mm TL, MZUSP 67874.

Fig. 3. Color pattern variation in *Gymnotus pantanal* from Pinheirinho and Jacutinga streams. (**a**) 209.8 mm TL (NUP 9311); (**b**) 181.0 mm TL (NUP 9311); (**c**) 150.0 mm TL (NUP 9312); (**d**) 125.0 mm TL (NUP 9312).

Morphological analysis

Counts and measurements followed Albert (2001) and Fernandes *et al*. (2005). When referring to *Gymnotus pantanal sensu stricto* or typical color pattern we followed Fernandes *et al*. (2005), who found a color pattern of obliquely-oriented thin pale pigment bands. The other specimens analyzed exhibited an atypical color pattern. Morphometric characters were summarized with a Principal Components Analysis (PCA; Pearson, 1901; Hotelling, 1933). Since the morphometric variables (Table 1) were strongly correlated, PCA was chosen as the most appropriate ordination technique to summarize variations. To choose the principal components for interpretation, we used the broken-stick model (Jackson, 1993).

To determine whether there were differences in the average scores of the axes retained for interpretation between fish with typical and atypical color patterns we used Student's t-test. The assumption of normality was evaluated with Shapiro-Wilk's test, whereas the assumption of homoscedasticity was evaluated with Levene's test. Mann-Whitney's U-test (similar non-parametric; Zar, 1999) was used when those assumptions

Table 2. Results of Principal Component Analysis (PCA). It is given, for each axis, the eigenvalues, the percent of variance explained and the broken-stick eigenvalues. For each variable is listed the eigenvector (loading or correlation). Number of specimens analyzed was: 25 of *G*. *pantanal sensu stricto* typical color pattern and 16 to *G*. *pantanal* atypical color pattern.

	Axis 1	Axis 2
Eigenvalue	3.652	2.195
Broken-stick eingenvalue	3.103	2.103
% of variance	30.433	18.294
Body depth/Total length	-0.295	-0.432
Body width/Total length	-0.084	0.480
Anal fin length/Body depth	-0.056	0.083
Preorbital length/Head length	-0.089	-0.340
Postorbital length/Head length	-0.297	0.223
Mouth width/Head length	-0.371	0.062
Interorbital length/Head length	-0.307	-0.011
Head depth/Head length	-0.375	0.164
Branchial opening/Head length	-0.368	0.135
Pectoral fin length/Head length	-0.342	-0.066
Preanal length/Head length	-0.358	0.193
Body width/Body depth	0.231	0.566

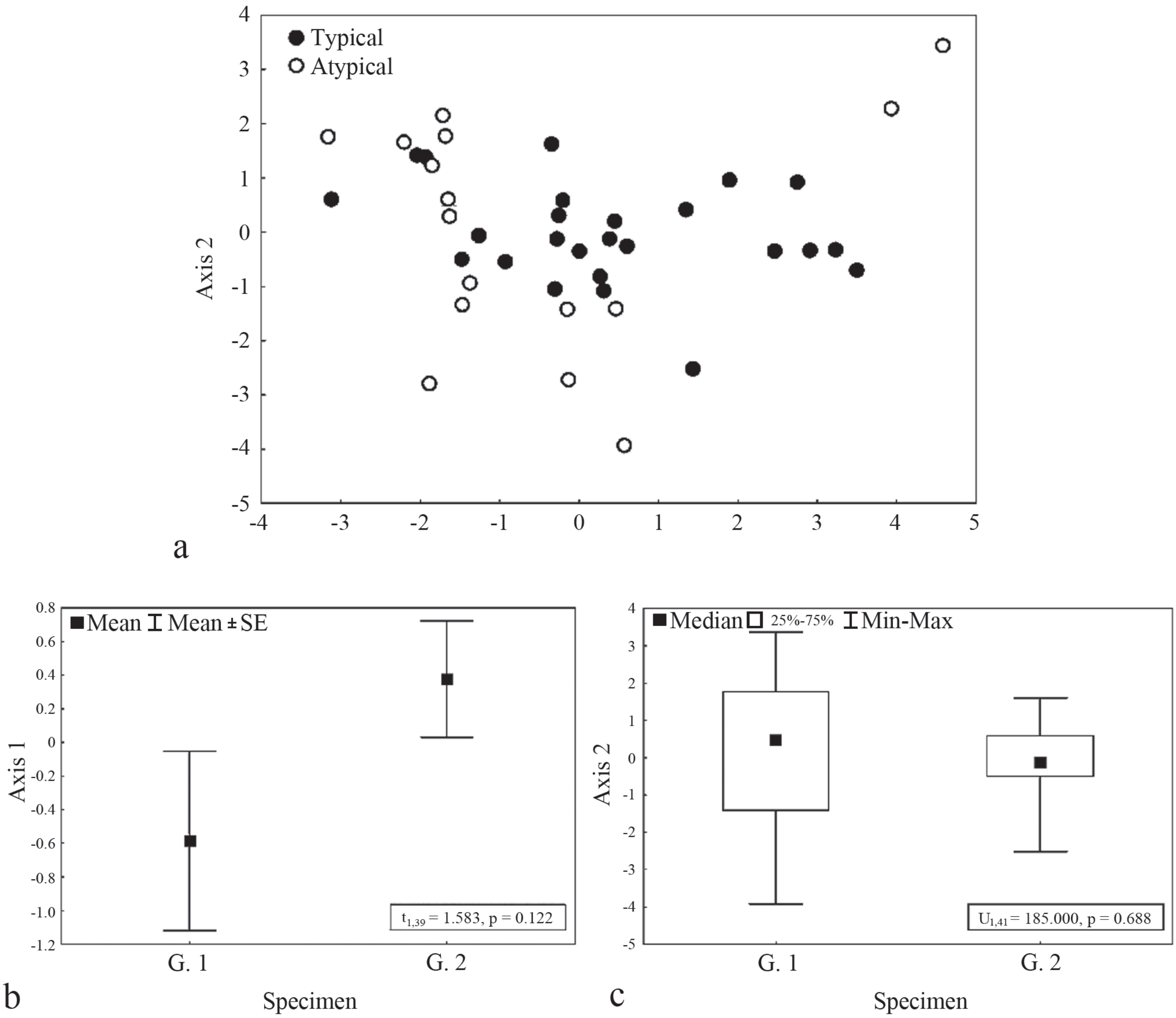

Fig. 4. Principal Component Analysis (PCA) ordination (**a**) and average values (± standard error) of the axes scores 1 (**b**) and 2 (**c**) from PCA ordination of morphometric characters for *Gymnotus pantanal* atypical color pattern (G. 1) and *Gymnotus pantanal sensu* stricto (G. 2).

were not met. Student's t-test and Mann-Whitney's U-test were performed in Statistica™ 7.0. The significance level adopted for all analysis was $p<0.05$.

Cytogenetic analysis

Chromosome analysis was carried out in 15 specimens with atypical color pattern (eight males and seven females). Mitotic chromosomes were obtained from cephalic kidney cells (Bertollo *et al*., 1978; Foresti *et al*., 1993). Fish were anesthetized with clove oil according to Henyey *et al*. (2002). Chromosomes were classified according to arm ratio (Levan *et al*., 1964) into two groups: meta-submetacentric (*m-sm*) and acrocentric (*a*).

Results

Thirty-three individuals were caught. Seventeen specimens were classified as typical, similar to the holotype (Fig. 2) and 16 as atypical (Fig. 3). Specimens of *G. pantanal* with atypical color pattern were observed in two sampling sites (Jacutinga and Pinheirinho). Total length varied from 113.4 to 248.0 mm (Table 1). The atypical color pattern was represented by a faint pigment band in the middle of the ventral region and another pigment band in the anal fin base (Fig. 3).

Mean values (min-max) of morphometric characters are presented in Table 1. Results from principal component analysis are summarized in the matrix of morphometric

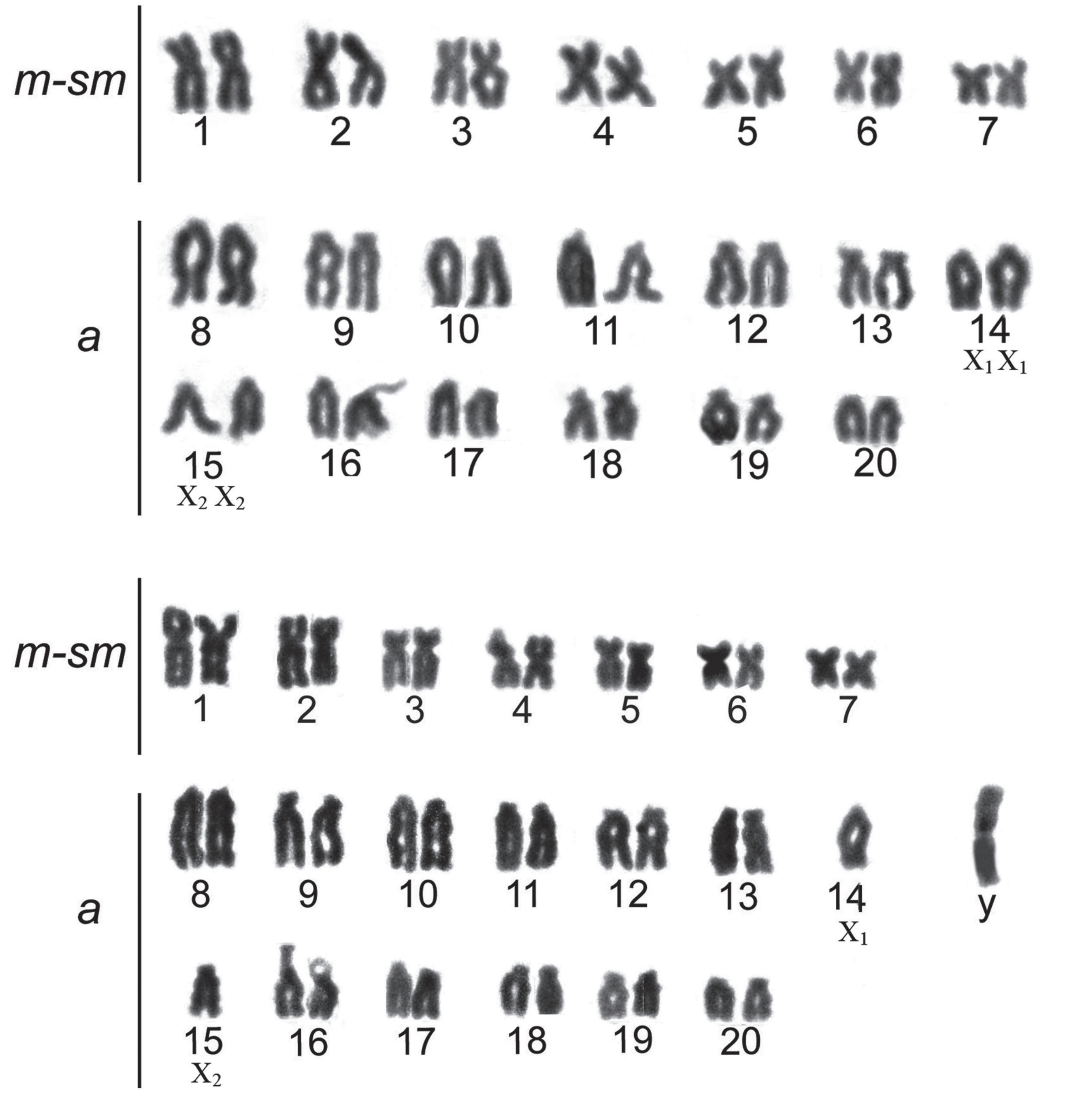

Fig. 5. Conventional Giemsa-stained karyotypes of *Gymnotus pantanal* (NUP 9312), atypical color pattern, female (above) and male (below) showing the X_1, X_2, and Y sex chromosomes.

characters. Two axes were chosen for interpretation (cumulative explained variance = 48.73%; Table 2).

There was no significant difference between the scores of the axes retained for interpretation (Fig. 4). Thus, there were no morphometric differences between the groups of specimens analyzed.

The cytogenetic results for all examined individuals showed that the diploid number was 40 for females and 39 for males. Females had 7 meta-submetacentric chromosome pairs and 13 acrocentric chromosome pairs; males had 7 meta-submetacentric chromosome pairs plus a large metacentric chromosome (chromosome Y), and 11 acrocentric chromosome pairs plus two non-homologous medium sized acrocentric chromosomes (chromosomes X_1 and X_2, Fig. 5).

Discussion

Color pattern is often the most important character for the diagnosis of *Gymnotus* species (*e.g.*, Albert *et al.*, 1999; Albert & Crampton, 2001, 2003; Fernandes *et al.*, 2005; Cognato *et* *al*., 2007; Maxime & Albert, 2009; Richer-de-Forges *et al*., 2009). Therefore, we thought at first that the atypical color pattern might suggest the existence of a new species. However, cytogenetic and morphological analyses did not corroborate this hypothesis. Moreover, many species of *Gymnotus* exhibit some variation in body shape and color patterns within and between populations (see Mago-Leccia, 1994; Albert & Miller, 1995; Albert *et al*., 1999).

Recently, Silva *et al*. (2010) observed variation in color pattern in specimens of *Trichomycterus iheringi* (Eigenmann, 1917) from the Rivers Itatinga and Claro, State of São Paulo, southeastern Brazil. These authors found two clearly distinct color patterns in this species and related this variation to body size and microhabitat preference.

Usually, morphological variations are associated with environmental, sexual, ontogenetic, and behavioral factors. This is very interesting, since we found *Gymnotus pantanal* specimens with typical and atypical color pattern syntopically in the streams Jacutinga and Pinheirinho, and only the typical color pattern in the stream Panambi. Daga (unpublished data) studied the effects of limnological and morphometric variables on the composition and structure of fish assemblage along a longitudinal gradient in the streams Pinheirinho, Jacutinga and Panambi, and observed a strong influence of urbanization in the distribution of the ichthyofauna. Variables such as total phosphorus, dissolved oxygen and conductivity were responsible for spatial changes in the fish assemblage. Moreover, the same author together with Gubiani *et al*. (2010) state that the stream Panambi is strongly affected by human impacts, as it receives both domestic and industrial sewage. With this, at the moment we cannot explain why color pattern varied only in the populations from the streams Pinheirinho and Jacutinga.

Additionally, the karyotype observed is identical to that of *G. pantanal* specimens studied by Fernandes *et al*. (2005) and by Silva & Margarido (2005). The occurrence of *G. pantanal* in the upper Paraná River basin was already described by Margarido *et al*. (2007) and by Graça & Pavanelli (2007). The formers used cytogenetic analysis to identify this invasive species, and pointed out the usefulness of this analysis for taxonomic diagnosis in this fish group due to its specific karyotype.

Therefore, our results corroborate the importance of integrating morphological and cytogenetic data to study the taxonomy of *Gymnotus*. External morphological data, mainly color pattern, may be misleading, as sometimes they result from mistakes in the identification of *Gymnotus* species.

Material examined. *Gymnotus pantanal*, Brazil, State of Mato Grosso do Sul. MZUSP 67874, holotype, 196.0 mm TL, female, Miranda River, 20 Jul 2000, near Miranda, 20º11'78"S 56º30'13"W. MZUSP 67875, paratype, 189.0 mm TL, and MZUSP 67876, paratype, 264.0 mm TL, Paraguay River, 22 Jul 2000, Corumbá, 18º59'81"S 57º39'24"W, State of Paraná. NUP 4554, 5, 195.3-234.8 mm TL, (all used in cytogenetic analyses by Silva & Margarido (2005) Paraná River (marginal lagoon), upper Paraná River basin. NUP 6044, 2, 152.3-203.5 mm TL, Água Queçaba stream, tributary to Pirapó River, upper Paraná River basin, 23º19'22"S 51º53'29"W. NUP 7934, 1, 152, Paracaí River, tributary to Paraná River, upper Paraná River basin, 13 Jul 2009, 23º39'30"S 53º55'10"W. NUP 9311, 5, 168.0-209.8 mm TL, Jacutinga stream, tributary to Marreco River, upper Paraná River basin, 6 Sep 2008, 24º42'56"S 53º46'21"W. NUP 9312, 11, 113.4-248.0 mm TL, (seven used in cytogenetic analyses), Pinheirinho stream, tributary of Toledo River, upper Paraná River basin, 6 Sep 2008, 24º44'05"S 53º42'55"W. NUP 9290, 17, 139.4-260.3, Jacutinga stream, tributary to Marreco River, upper Paraná River basin, 6 Sep 2008, 24º42'56"S 53º46'21"W.

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