

## A new *Gymnotus* (Teleostei: Gymnotiformes: Gymnotidae) from the Pantanal Matogrossense of Brazil and adjacent drainages: continued documentation of a cryptic fauna

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### Abstract

Here we describe a new species of *Gymnotus*, *G. pantanal* n. sp., from the Pantanal Matogrossense of Brazil, using morphological, cytogenetic, and molecular data. Specimens ascribed to the new species are also known from areas downstream in Paraguay, and from the adjacent Guaporé basin of Bolivia. The new species most closely resembles *G. anguillaris* in possessing an elongate body, slender profile, long body cavity, and shorter head than other congeners. The new species also resembles *G. anguillaris* in the presence of pale narrow bands restricted to the area below the lateral line on the anterior half of the body. The new taxon differs from *G. anguillaris* in possessing more narrowly set eyes, a wider and deeper head, a larger branchial opening, longer pectoral fins with more fin rays, and fewer pored posterior lateral-line scales. The new species inhabits rooted grasses and floating macrophytes in small creeks and along the banks of larger blackwater rivers. Populations are found syntopically with *G. inaequilabiatus* and *G. sylvius*. Compared with these species, the new species exhibits a distinct combination of microsatellite DNA amplification patterns, and chromosomal and external features. These results confirm earlier studies showing the power of a multidisciplinary approach to characterizing the enormous and often cryptic diversity of Neotropical fishes.

**Key words:** Gymnotiformes, microsatellite, chromosome, cryptic diversity

**Portuguese Abstract**

Descrevemos aqui uma nova espécie de *Gymnotus*, *G. pantanal* n.sp., do Pantanal Matogrossense do Brasil, usando dados morfológicos, citogenéticos e moleculares. Espécimes atribuídos à nova espécie são também conhecidos de áreas a jusante no Paraguai, e da adjacente bacia do Rio Guaporé na Bolívia. A nova espécie apresenta maior semelhança com *G. anguillaris*, possuindo um corpo alongado, perfil delgado, cavidade do corpo longa, e a cabeça menor do que a de outros congêneres. A nova espécie ainda se assemelha a *G. anguillaris* pela presença de bandas pálidas estreitas restritas à área abaixo da linha lateral na metade anterior do corpo. O novo táxon difere de *G. anguillaris* por possuir olhos localizados mais próximos, uma cabeça mais larga e mais alta, uma abertura branquial mais ampla, nadadeiras peitorais mais longas com maior número de raios, e um menor número de escamas perfuradas, na região posterior da linha lateral. A nova espécie habita regiões com gramíneas e áreas com macrófitas flutuantes em pequenos riachos e ao longo de margens de rios maiores de águas escuras. As populações são encontradas em sintopia com *G. inaequilabiatus* e *G. sylvius*. Comparada com essas duas espécies, a nova espécie exibe uma combinação distinta de padrões de amplificação de microssatélites, de cromossomos e de características externas. Esses resultados confirmam estudos anteriores mostrando o poder da abordagem multidisciplinar na caracterização da enorme e muitas vezes críptica diversidade dos peixes neotropicais.

**Introduction**

*Gymnotus* (L.) is a monophyletic group of gymnotiform fishes that is readily recognizable by the presence of a superior mouth with a prognathous lower jaw, a fleshy pad of electroreceptor organs and support tissues over the tip of the snout and mandible, a pair of dorsally oriented pipe-shaped anterior nares partially or entirely included within the gape, a ventrally curved rictus, the lateral position of the eyes on the head (at a horizontal with the gape), numerous long rami of the posterior lateral line extending ventrally on the caudal portion of the body, and a very long body cavity with 31–48 precaudal vertebrae (Albert 2001; Albert *et al.* 2005). Most *Gymnotus* species possess obliquely oriented bands of dark and light pigments along the length of the body from which they derive the English common name “banded knife-fish.”

*Gymnotus* species extend from the Pampas of Argentina (36 °S) to Chiapas, Mexico (18 °N) and are known from the continental waters of all South and Middle American countries except Chile and Belize (Albert 2001). Many species of *Gymnotus* exhibit sizeable variation in body shape and color patterns within and between populations (Mago-Leccia 1994; Albert & Miller 1995; Albert *et al.* 1999). Since these features are also used to recognize *Gymnotus* species, much of the diversity in the group remains undocumented and many undescribed species exist in museum collections and in the wild (Nelson, 1994, Albert *et al.* 1999; Albert 2001). There are currently 31 species of *Gymnotus* recognized (Albert *et al.* 2005). As for many Neotropical freshwater fishes, species diversity of this group is greatest in the Amazon basin where 15 species are known, 10 of which inhabit v-rzea whitewater floodplains (Albert & Crampton 2001, Crampton *et al.* 2003). Fewer

data are available on species diversity, distributions and population structure of this group in other Neotropical basins (Fernandes-Matioli *et al.* 2000).

In central and southeastern Brazil many specimens are ascribed to *G. carapo* L., the type locality of which is in Surinam (Albert 2001). Three other *Gymnotus* species have been recognized from central and southeastern Brazil: *G. pantherinus* (Steindachner) from the coastal drainages of southeastern, Brazil, *G. inaequilabiatus* (Valenciennes) from the Parana-Paraguay basin and some coastal drainages of Uruguay and southeastern Brazil, and *G. sylvius* (Albert *et al.* 1999) from coastal drainages of São Paulo State and the Rio Paraná basin.

Here we describe a new species discovered by one of us (FMCF) as part of a survey of population structure and genealogical/coalescent analysis of *Gymnotus* from the Pantanal Matogrossense, Brazil. The new species most closely resembles *G. anguillaris* Hoedeman, originally described from Surinam. *Gymnotus anguillaris* was distinguished from syntopic specimens of *G. carapo* by a cylindrical body shape and a restriction of the pale oblique bands to the caudal portion of the body. Specimens with this characteristic body shape and color pattern have been subsequently collected from the Orinoco, Amazon, and Parana river basins (Ellis, 1913; Albert 2001; Albert & Crampton, 2001). Compared with its sympatric congeners, the new species exhibits a distinct combination of morphological, genetic, cytogenetic and external features, including some body proportions, chromosomal organization and specific microsatellite amplification patterns in the genome. These results extend those of earlier studies showing the power of a multidisciplinary approach to characterizing the enormous and often cryptic diversity of Neotropical fishes (Murphy & Thomerson 1999; Hrbek & Larson 1999; Albert *et al.* 1999; Albert & Crampton 2001).

## Materials and Methods

Specimens of the type series were collected from the Rio Miranda and Rio Paraguay basins, in the Pantanal Matogrossense, state of Mato Grosso do Sul, Brazil. Individuals were analyzed using morphological, cytogenetic and molecular approaches. Institutional abbreviations are as listed in Leviton *et al.* (1985), with the addition of FML, Fundacion Miguel Lolli, Tucuman, Argentina, and LGP, Laboratório de Ictiogenética, Departamento de Biologia, Universidade de São Paulo, São Paulo, Brazil.

Measurement protocols follow Albert (2001). Osteological data were taken from cleared and stained specimens using the enzyme technique of Taylor and Van Dyke (1985). We used standardized microdissection methods for small teleosts (Weitzman 1962) and follow Fink and Fink (1981) and Albert (2001) for morphological nomenclature. Body size is represented by total length in millimeters. Specimens in which the caudal appendage was obviously damaged and not, or only partially, regenerated were excluded from measurements of total length. Descriptions of meristic features apply to specimens of all sizes, both juveniles and adults. Morphometric, osteological, and pigmentation character-

istics apply only to morphologically (as opposed to reproductively) mature specimens unless otherwise stated. Size at morphological maturity was estimated as the asymptotic value of head length within a range of values due to natural variance in the mature population (e.g., Crampton et al., 2005, fig. 2). Counts of precaudal vertebrae and anal-fin rays were taken from radiographs or cleared and stained specimens. The number of precaudal vertebrae includes the 5 in the Weberian Apparatus; number of precaudal vertebrae is used here as a proxy for body-cavity length (Albert & Fink 1996).

Chromosome preparations follow Foresti *et al.* (1981) with the modification that specimens were injected with 0.02% colchicine (0.50ml/100g bodyweight) 50 min before sacrifice. Cephalic kidney was extracted and minced in a 0.075M KCL solution, placed in an incubator at 37 °C for 27 min. Six ml of methanol: acetic acid (3:1) solution was added and centrifuged (1,200 rpm) for 10 min. The supernatant was discarded and the cell pellet was fixed three times in a methanol: acetic acid (3:1) solution and centrifuged (1,200 rpm) for 6 min. The pellet was resuspended in fresh fixative and dropped on heat slides (60 °C). The slides were stained with a 3% Giemsa staining solution. The nucleolus organizer regions (NORs) were silver stained according to Howel and Black (1980), and C-bands were obtained according to Sumner (1972). Karyograms were analyzed for centromeric placement and arm ratios. The chromosomes were arranged in decreasing order of size in two groups: metacentric/submetacentric (M/SM) and subtelocentric/acrocentric (ST/A).

The molecular marker *micro11* exhibits species-specific amplification patterns via SPAR-PCR (single primer amplification reaction-polymerase chain reaction) in Gymnotidae (Fernandes-Matioli *et al.* 2000). DNA samples were extracted from scale and fin tissues preserved in 96% ethanol. DNA was isolated by the standard phenol: chloroform protocol (Sambrook *et al.* 1989). The tetranucleotide primer (GGAC)<sub>4</sub> was used in the amplifications. The PCR conditions were: 1 ng of DNA sample was amplified in a final volume of 30 µL containing 10mM Tris.HCl, pH 8.4, 0.5% nonidet P-40, 50mM KCl, 5.0 mM MgCl<sub>2</sub>, 100 µM each of dNTP, 5 pmol primers, 1.25 units of *Taq*DNA polymerase (Life Technologies). Amplifications were performed in an Eppendorf Mastercycler Gradient machine for 30 cycles. The cycles consisted of 45 s at 94 °C, 60 s at 53 °C and 60 s at 72 °C. All products were analyzed on 1.4 % agarose gels stained by ethidium bromide.

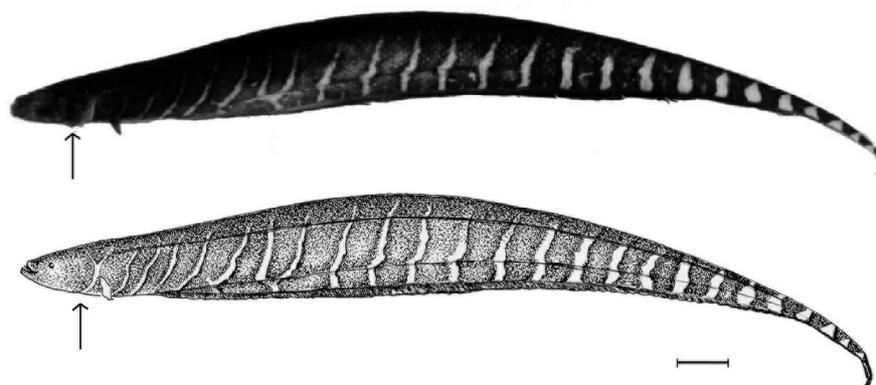
## Systematic Descriptions

### *Gymnotus pantherinus* species-group Albert 2001

#### *Gymnotus pantanal* new species

(Fig. 1)

*Holotype*: MZUSP 67874, female, Brazil, Mato Grosso do Sul State, Rio Miranda, 196 mm, 20 July 2000, near Miranda, 20°11' 78" S, 56°30' 13" W, F. M. C. Fernandes.



**FIGURE 1.** Top: Holotype (MZUSP 67874) of *G. pantanal* n. sp. in left lateral view. Bottom: schematic illustration of *G. pantanal* n. sp. in left lateral view. Arrows indicate position of anus. Note very narrow pale interbands in the anterior portion of the body. Scale bar = 1 cm.

*Paratypes*: 2 spec. MZUSP 67875, 189 mm, and MZUSP 67876, 264 mm, Brazil, Mato Grosso do Sul State, Rio Paraguay, 22 July 2000, Corumbá, 18°59' 81" S, 57°39' 24" W, F. M. C. Fernandes.

*Nontypes*: **Bolivia**: UF 82146 (1), Santa Cruz, near Concepcion, Rio Blanco, 153 mm, 1990.06.16. **Brazil**: MZUSP 67876 (1), Mato Grosso do Sul, Rio Paraguay, 18°59'81"S, 57°39'24"W, 251 mm, 22 July 2000. MZUSP 67875 (1), Mato Grosso do Sul, Rio Paraguay, 18°59'81"S, 57°39'24"W, 192 mm, 22 July 2000. **Paraguay**: NRM 42830 (1), Rio Parana, 240 mm, 1998.03.15. NRM 42397 (1), Rio Paraguay, 171 mm, 1998.03.25. UF 38173 (1), Dept Cochabamba, Province Chapare, Rio Espiritu, Chapare-Mamore drainage, brook at Villa Tunari, elevation 350 m., 192 mm, 1982.XII.12. UMMZ 206080 (21), Arroyo in Parque Nacional Ybycui, Rio Paraguay, 82–260 mm, 1979.VI.20.

*Diagnosis*: *Gymnotus pantanal* differs from other members of the *G. pantherinus* species-group (except *G. anguillaris*) 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. *Gymnotus pantanal* further differs from other members of the *G. pantherinus* species-group in possessing a wider head (72–77 vs. 52–69% head length). *Gymnotus pantanal* further differs from *G. anguillaris* in possessing more narrowly set eyes (37–41 vs. 43–56% head length), a deeper head (66–74 vs. 60–65% head length), larger branchial openings (38–43 vs. 29–36% head length), longer pectoral fins (51–56 vs. 42–50% head length), more pectoral-fin rays (mode 17 vs. 16), and fewer pored posterior lateral-line scales (to first ventral ramus: 47–58 vs. 58–62; total: 102–114 vs. 124–130).

*Description*: Fig. 1 illustrates body shape and pigment patterns. Morphometric and meristic data for specimens in type series and additional lots in Table 1. Size up to 251 mm. No known sexual dimorphism. Adult body proportions attained at about 120 mm

total length. Size at sexual maturity unknown. Adult body shape subcylindrical, ratio body width/depth 0.70–0.81. Body profile slender, body depth 8.0–9.5% total length. Head length moderate, 8.6–9.3% total length. Snout length moderate, 34–37% head length. Mouth width moderate, 44–48% head length. Preanal distance moderate, 82–93% head length. Anal-fin long, 78–82% total length. Scales present on entire post-cranial portion of body from nape to caudal appendage. Scales above lateral line large, (7–8, mode 8). Scales cycloid, ovoid. Scales over anal-fin pterygiophores large, (mode) 5–6 rows.

**TABLE 1.** Morphometric and meristic data for *Gymnotus pantanal* n. sp. Data for adult specimens (> 120 mm TL). Total length and head length expressed in mm. Anal-fin length, body depth, and body width as percentage total length. Other measurements as percentage head length. AVG = mean for morphometric data; median for meristic data. N values less than 13 due to damaged specimens or limited radiography.

Measurement	MIN	MAX	N	AVG
<b>Morphometrics</b>				
Total length (mm)	84	251	13	-
Head length (mm)	9.7	21.5	13	-
Head length	8.6	10.1	11	9.1
Preorbital length	34	37	13	35
Postorbital length	59	62	13	60
Mouth width	44	48	13	46
Interorbital length	37	41	13	39
Head depth	66	74	13	70
Head width	72	77	13	74
Branchial opening	38	43	10	40
Body depth	8.0	9.5	13	8.8
Body width	6.0	7.2	13	6.5
BW / BD	0.70	0.81	13	0.74
Pectoral fin length	51	56	13	53
Preanal length	79	96	13	88
Anal-fin length	78	81	12	80
<b>Meristics</b>				
Color bands	7	25	12	13
Pectoral fin rays	16	18	11	12
Scales above lateral line	7	8	13	8
Scales to first ramus	47	58	13	54
Scales to last lateral-line pore	102	114	6	108
Precaudal vertebrae	35	38	6	37
Anal-fin rays	235	280	6	258

Gape size in mature specimens large, extending to or beyond posterior nares. Mouth position superior, rictus decurved. Eye position below horizontal with front of mouth. Anterior narial pore partially or entirely included within gape. Circumorbital series ovoid. Maxilla orientation vertical. Maxilla rod- or paddle-shaped with straight ventral margin. Dorsoposterior laterosensory ramus of preopercle with single superficial pore. Cranial fontanelles closed in juveniles and adults. Anterior margin of frontal straight, continuous with margins of adjacent roofing bones. Frontal postorbital process narrow, less than two times width of supraorbital canal. Frontal broad, its width at the posterior articulation of the infraorbital series subequal to that of parietal. Pectoral fin broad, with 15–19 (mode 17) rays. Anterior limb of cleithrum long, more than 1.8 times ascending limb. Cleithrum anterior notch absent. Cleithrum without large facet for insertion of muscle from supracleithrum. Body cavity long, with 35–38 (mode 37) precaudal vertebrae. Rib 5 broad, with a large medial triangular shelf. Hemal spines present. Displaced hemal spines absent. Anal fin of moderate length, with 217–260 rays. Multiple anal-fin ray branching posterior to rays 10–17. Lateral-line ventral rami 7–12. Lateral-line dorsal rami absent in adults. Length anal-fin pterygiophores equal to or longer than hemal spines. Caudal appendage long, more than 0.5 time pectoral-fin length in undamaged and unregenerated specimens. Single hypaxial electric organ, extending along entire ventral margin of body. Two to three (mode 3) rows of electroplates near caudal insertion of anal fin. Electric organ discharge not known.

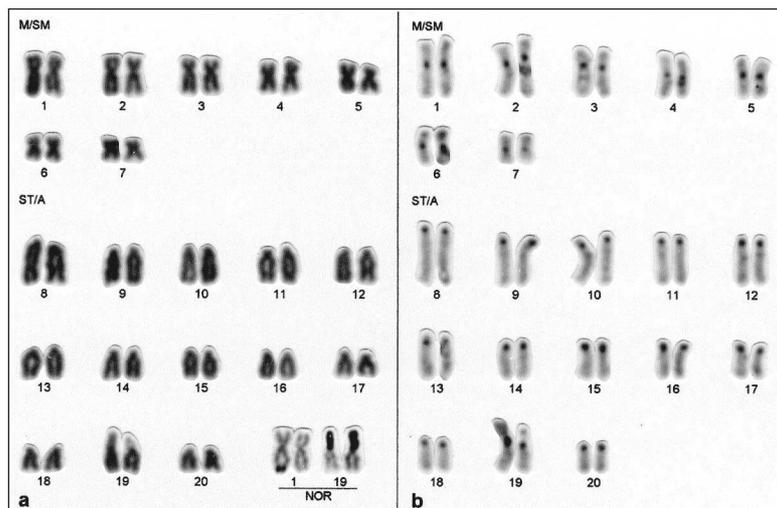
*Color in alcohol:* Ground color of body dark brown. All juveniles and some adults (TL more than 160 mm) with 21 to 26 obliquely oriented, thin pale-yellow bands with wavy irregular margins on ventrolateral surface, extending from tip of tail to pectoral-fin base. Some subadults and most adults with fewer pale bands. Band appearance variable in shape, width, arrangement, and number, both on and among individuals. Band-interband margins irregular and wavy. Dark bands evenly pigmented, or partially divided ventrally (inverted Y-shaped) at middle to posterior portions of body. Dark bands four times as broad as pale bands on anterior half of body. In adults pale bands rarely branched, and never extending above lateral line on anterior half of body. Interband contrast increases ventrally and caudally; more pronounced in smaller specimens. Anterior 80% of dorsum (anterior to anal-fin clear patch) without banding. Three bands from either side meet on ventral midline, between the anus and anal-fin origin. One band lies posterior to last anal-fin ray.

Head not banded or blotched; ground color dark brown dorsally grading to lighter brown ventrally, without freckles and with numerous speckles distributed over branchiostegal membranes and ventral surface of head. Pectoral-fin rays brown or gray, inter-radial membranes hyaline. Anal-fin membrane uniformly light brown to dusky gray.

*Comparisons with other species.* In terms of salient features of color pattern and general body proportions, *G. pantanal* most closely resembles *G. anguillaris* from which it differs by character states provided in the Diagnosis. *Gymnotus anguillaris* and *G. pantanal*

can be differentiated from other members of the *G. pantherinus* species-group by the following unique combination of characters: no pale band at nape, pale bands not extending above lateral line on anterior half of body, adult body size greater than 200 mm total length, fewer ventral lateral-line rami (7–18 vs. 19–30), fewer anterior anal-fin rays without multiple branching (10–17 vs. 18–26), and a single row (vs. two rows) of conical teeth along outer margin of dentary.

On morphometric grounds, *G. pantanal* can be separated from sympatric congeners (i.e., *G. carapo*, *G. inaequilabiatus*, and *G. sylvius*) of similar size (130–250 mm TL) by a shorter head (head length 8.6–10.1% vs. 10.5–14.0 total length) and a more slender body (BD 9.0–9.5 vs. 10.0–13.0% total length). Among species of *Gymnotus*, *G. pantanal* most closely resembles *G. anguillaris* from Surinam, in terms of morphometric, meristic, and color variables. *Gymnotus pantanal* shares several meristic counts with other species from southern Brazil, including the number of the pectoral-fin rays (16–18) and number of scales above the lateral line at midbody (7–8). Juvenile specimens of *G. pantanal* also share 21–26 oblique bands (or band pairs) with sympatric congeners and *G. anguillaris* from Surinam.



**FIGURE 2.** Karyotype of *Gymnotus pantanal* n. sp., MZUSP 67874,  $2n=40$ . a) Chromosomes stained by Giemsa, showing seven pairs of metacentric/ submetacentric (M/SM) and 13 pairs of subtelocentric/acrocentric (ST/A) chromosomes. In inset, the NOR-bearing chromosomes, pairs number 1 and 19. b) C-bands distribution. Note presence of constitutive heterochromatin in the pericentromeric region of all chromosomes.

*Karyological features:* Diploid number of  $2n=40$  chromosomes, arranged in seven pairs of metacentric/submetacentric and 13 pairs of subtelocentric/acrocentric chromosomes. Chromosome pair 19 with a secondary constriction on the short arms, at the site of active NORs (evidenced after silver staining). A third active NOR is also present in one of

the homologues of the larger pair of metacentric/ submetacentric on the terminal region of the long arm. The C-bands evidence the constitutive heterochromatin distribution in the pericentromeric region of all chromosome pairs. The karyological features observed in the holotype (MZUSP 67874) are illustrated in Fig. 2 and summarized in Table 2. According to data available in the literature, *G. pantanal* shares the diploid number of  $2n=40$  chromosomes with *G. sylvius*. However, the chromosome morphology differs greatly between these two species. *Gymnotus pantanal* possesses seven pairs of metacentric/submetacentric and 13 pairs of subtelocentric/acrocentric chromosomes, whereas *G. sylvius* possesses 14 pairs of metacentric, 5 pairs of submetacentric and 1 pair of subtelocentric/acrocentric chromosomes. The occurrence of constitutive heterochromatin in the pericentromeric region of the chromosomes is shared with the other species from southern Brazil (see Fernandes-Matioli *et al.* 1998). *G. pantanal* also possesses a unique cytological characteristic among the species chromosomally characterized; the presence of 3 active NORs in the metaphase plates (only two active NORs is observed in the other species).

**TABLE 2.** Cytogenetic and molecular data for five species of *Gymnotus* from southern Brazil.

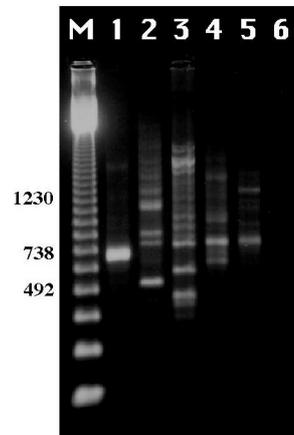
Species	$2n^a$	Formula <sup>a,c</sup>	NOR <sup>a</sup>	<i>micro11</i> bands <sup>b</sup>	molecular size (~base pairs) <sup>b</sup>
<i>G. carapo</i>	54	44M,8SM,2ST/A	2	2	800/1,476
<i>G. sylvius</i>	40	28M,10SM,2ST/A	2	1	650
<i>G. pantherinus</i>	52	38M,8SM,6ST/A	2	4	530/810/861/1,150
<i>G. inaequilabiatus</i>	52	40M,10SM,2ST/A	2	4	400/600/810/1,968
<i>G. pantanal</i>	40	14M/SM,26ST/A	3	2	800/1,370

a. Data from Fernandes-Matioli *et al.* (1998), except for *G. pantanal*

b. Data from Fernandes-Matioli *et al.* (2000), except for *G. pantanal*

c. M, metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric.

*Molecular characterization:* Amplification of the microsatellite marker *micro11* (Fernandes-Matioli *et al.* 2000) using the SPAR-PCR technique resulted in a unique pattern of two amplified bands (fragments) of relatively high molecular weight, 800 base pairs (bp) and 1,370 bp. This pattern was observed in ten individuals analyzed (Table 2, Fig. 3). The molecular marker *micro11* exhibits species-specific patterns among members of the Gymnotidae (Fernandes-Matioli *et al.* 2000). The *micro11* pattern observed in all *G. pantanal* individuals analyzed, with two bands of relatively high molecular size (800 bp and 1,370 bp, Table 2), differs conspicuously from its congeners (Fig. 3). Amplified fragments with high molecular weight, albeit with different size, are also observed in *G. carapo* (one of two bands with 1,476 bp), in *G. inaequilabiatus* (one of four bands with 1,968 bp) and in *G. pantherinus* (one of four bands with 1,150 bp). The molecular *micro11* pattern observed in *G. pantanal* is unique among congeners in southern Brazil.



**FIGURE 3.** PCR products showing the *micro11* patterns of five species of *Gymnotus* obtained using  $(GGAC)_4$  as primer. M, standard molecular marker (123 DNA ladder, GibcoBRL); 1, *G. sylvius* (G375) from Paraibuna, São Paulo; 2, *G. pantherinus* (G135), from Engenheiro Marsilac, São Paulo; 3, *G. inaequilabiatus* (D16), from Rio Claro, São Paulo; 4, *G. carapo* (G204), from Porto Primavera, São Paulo; 5, *G. pantanal* n. sp. (G302), from Corumbá, Pantanal Matogrossense, Mato Grosso do Sul; 6, negative control.

*Distribution:* Known from the Parana-Paraguay system of Brazil and Paraguay, and the Rio Chapare-Mamoré of Bolivia.



**FIGURE 4.** Drainage map of part of South America showing geographic distribution of *G. pantanal* n. sp. and similar species. *G. anguillaris sensu stricto* (stars); *G. cf. anguillaris* (squares); *G. pantanal* n. sp. (triangles). Some symbols represent more than one locality or lot of specimens.

*Etymology*: The specific epithet *pantanal* from the Pantanal Matogrossense of Brazil, the hydrological region of the type locality. A noun in apposition.

## Discussion

Among congeners, *G. pantanal* most closely resembles *G. anguillaris* from the area of the type locality in Surinam in aspects of coloration, and also in several morphometric and meristic features associated with a highly elongate body (i.e., body slender, body cavity long, and relative head length short). In combination, these particular features are known to have diagnostic value for differentiating other *Gymnotus* species living in sympatry, under conditions when species-identities are well established from additional electric signal, chromosomal, or molecular data (Albert *et al.* 1999; Albert & Crampton, 2001). *Gymnotus pantanal* also differs from *G. anguillaris* in several features known to have diagnostic value at the species level within *Gymnotus*: i.e., interorbital distance, size of the branchial opening, pectoral-fin length, number of fin pectoral-fin rays, and lateral-line scale counts.

At present it is not possible to determine whether phenotypic intergrades occur between *G. pantanal* and *G. anguillaris*. Specimens of the *G. anguillaris* species-complex are absent from collections from the central and eastern Amazon (Crampton, 1998; Albert & Crampton, 2001), including from the extensive INPA collections of these areas (JSA and WGRC, pers. obs.). Undescribed populations resembling *G. anguillaris* are known from tributaries of the Napo and Madeira rivers in the Amazon basin (Fig. 4), each possessing distinct phenotypes. The morphological, cytogenetic, and molecular data reported here indicate that the populations described here as *G. pantanal* represent a distinct species (i.e., a separately evolving evolutionary lineage) rather than a geographic variant of *G. anguillaris*. This hypothesis will be tested with future collections of the *G. pantherinus* species-group from across its range, and by comparisons of molecular sequence data.

## Materials Examined

Additional lots, and lots with revised identifications, examined since Albert (2001), of *G. anguillaris* and of *G. inaequilabiatus*. are arranged alphabetically by species, country, and institutional abbreviations. Catalogue numbers followed in parentheses by numbers of specimens, and when data are available, total length range in millimeters, locality, and date of capture. HT, Holotype; PT, paratypes; uncat., uncatalogued. *cf.* (Latin *confere*) indicates species closely resembling, but not conforming to, a published diagnosis.

*Gymnotus anguillaris*. — **British Guyana**: BMNH 1972.10.17.371–396 (26), 140–305 mm, Potaro River, Amatuk, Essequibo River, 1972. **French Guiana**: NRM 28326 (2),

Crique Soumouro Kourou, 1994.III.20. **Surinam**: UMMZ 190413 (3), 131–289 mm, Maka Creek, Lawa River, Marowijne, 1971.04.22. ZMA 100338 (HT), 228 mm, Marowijne, Lawa River, Coropina creek, station 18, 1956. V.20. ZMA 100338a (PT), 236 mm, same collection data as HT. ZMA 105930 (4), 255–302 mm, Maka Creek, Lawa River, Marowijne, 21.IV.1967.

*Gymnotus cf. anguillaris*. — **Brazil**: INPA 11553 (1), 111 mm, Mato Grosso, Ig. do aeroporto. Cidade de Humboldt. Rio Aripuana, 1976.XI.09. INPA 11556 (1), 185 mm, Mato Grosso, Ig. do aeroporto. Cidade de Humboldt. Rio Aripuana, 1976.XI.09. INPA 6388b (1) 348 mm, Mato Grosso, Rio Aripuana. Regiao do Castanhal, 1976.VIII.22. INPA 6407b (4) 160–190 mm, Mato Grosso, Ig. do aeroporto. Cidade de Humboldt, Rio Aripuana, 1976.XI.09. INPA 6408b (9) 96–223 mm, Mato Grosso, Ig. acima da cachoeira, Rio Aripuana, 1976.XI.14.

*Gymnotus inaequilabeatus*. — **Brazil**: MCP 7155 (1), 254 mm, Rio Maquine, Osorio, 1984.IX.26. MZUSP 46001 (1), 998 mm, Porto Primavera, Rio Parana, São Paulo, 1993.VIII. MZUSP 51667 (1), Paraibo do Sul, Jacarei, São Paulo, 1993.I.24. MZUSP 51268 (1), c. 370 mm, Rio Capivara, affluent do Rio Paranapanema, São Paulo, 1994.III. USNM 1643 (1), 791 mm, Rio Paraguay.

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### Literature Cited

- Albert, J.S. (2001) Species diversity and phylogenetic systematics of American knifefishes (Gymnotiformes, Teleostei). *Miscellaneous Publications of the Museum of Zoology, University of Michigan*. 190, 1–127.
- Albert, J.S. & Crampton W.G.R. (2001) Five new species of *Gymnotus* (Teleostei: Gymnotiformes) from an Upper Amazonian floodplain, with descriptions of electric organ discharges and ecology. *Ichthyological Exploration of Freshwaters*, 12(3), 241–226.
- Albert, J.S. & Crampton, W.G.R. (2003) Seven new species of the Neotropical electric fish *Gymnotus* (Teleostei, Gymnotiformes) with a redescription of *G. carapo* (Linnaeus). *Zootaxa*, 287: 1–54.
- Albert, J.S. & Fink, W.L. (1996) *Sternopygus xingu*, a new species of electric fish from Brazil (Teleostei: Gymnotoidei), with comments on the phylogenetic position of *Sternopygus*. *Copeia*

- 1996, 85–102.
- Albert, J.S. & Miller, R.R. (1995) *Gymnotus maculosus*, a new species of electric fish from Middle America (Teleostei: Gymnotoidei), with a key to the species of *Gymnotus*. *Proceedings of Biological Society of Washington*, 108(4), 662–678.
- Albert, J.S., Fernandes-Matioli, F.M. C. & Almeida-Toledo, L.F. (1999) A new species of *Gymnotus* (Gymnotiformes, Teleostei) from Southeastern Brazil: towards the deconstruction of *Gymnotus carapo*. *Copeia* 1999(2), 410–421.
- Albert, J.S., Crampton, W.G.R., Thorsen, D. H. & Lovejoy, N.R. (2005) Phylogenetic systematics and historical biogeography of the Neotropical electric fish *Gymnotus* (Teleostei: Gymnotiformes). *Systematic and Biodiversity*, 2(4), 375–417.
- Crampton, W.G.R. (1998) Effects of anoxia on the distribution, respiratory strategies and electric signal diversity of gymnotiform fishes. *Journal of Fish Biology*, 53, 307–330.
- Crampton W.G.R., Lovejoy, N.R. & Albert, J.S. (2003) *Gymnotus ucumari*: a new species of Neotropical electric fish from the Peruvian Amazon (Ostariophysi: Gymnotidae), with notes on ecology and electric organ discharges. *Zootaxa*, 277, 1–18.
- Crampton, W.G.R., Thorsen, D.H. & Albert, J.S. (2005) Three new species from a diverse sympatric assemblage of *Gymnotus* (Gymnotiformes: Gymnotidae) in the lowland Amazon basin, with notes on ecology. *Copeia*, (1), 82–99.
- Ellis, M.M. (1913). The gymnotid eels of tropical America. *Memoires Carnegie Museum*, 6, 109–195.
- Fernandes-Matioli, F.M.C., Marchetto, M.C.N., Almeida-Toledo, L.F. & Toledo-Filho, S.A. (1998) High intraspecific karyological conservation in four species of *Gymnotus* (Pisces: Gymnotiformes) from Southeastern Brazilian basins. *Caryologia*, 51, 221–234.
- Fernandes-Matioli, F.M.C., Matioli, S.R & Almeida-Toledo, L.F. (2000) Species diversity and geographic distribution of *Gymnotus* (Pisces: Gymnotiformes) through analysis of nuclear (GGAC)<sub>n</sub> microsatellites. *Genetics and Molecular Biology*, 23, 803–807.
- Fink, S.V. & Fink, W. L. (1981) Interrelationships of the ostariophysan fishes (Teleostei). *Zoological Journal of the Linnean Society, London*, 72, 297–353.
- Foresti, F., Almeida-Toledo, L.F. & Toledo-Filho, S.A. (1981) Polymorphic nature of nucleolus organizer regions in fishes. *Cytogenetics and Cell Genetics*, 31, 137–144.
- Hoedeman, J. J. (1962) Notes on the ichthyology of Surinam and other Guianas. 9. New records of gymnotid fishes. *Bulletin of Aquatic Biology*, 53–60.
- Howell, W. M. & Black, D. A. (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: I — step method. *Experientia*, 36, 1014–1015.
- Hrbeck, T. & Larson, A. (1999). The evolution of diapause in the killifish family Rivulidae (Atherinomorpha, Cyprinodontiformes): A molecular phylogenetic and biogeographic perspective, *Evolution* 53, 1200–1216.
- Leviton, A.E., Gibbs, R.H., Heal, E. & Dawson, C.E. (1985) Standards in herpetology and ichthyology. Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia* 1985, 802–832.
- Mago-Leccia, F. (1994) *Electric fishes of the continental waters of America*. Biblioteca de la Academia de Ciencias Fisicas, Matematicas, y Naturales. Caracas, Venezuela, 29, 1–206.
- Murphy, W.J. & Thomerson, J.E. (1999) Phylogeny of the Neotropical killifish family Rivulidae (Cyprinodontiformes, Aplocheiloidei) inferred from mitochondrial DNA sequences, *Molecular Phylogeny and Evolution*, 13, 289–301.
- Nelson, J.S. (1994) *Fishes of the World*. 3 ed., John Wiley and Sons, Inc. U.S.A., 600 pp.
- Sambrook, J., Fritsch, E.F. & Maniatis T. (eds.). (1989) *Molecular cloning, a laboratory Manual*. 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Taylor, W.R. & Van Dyke, G.C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, 9, 107–119.

Weitzman, S.H. (1962). The osteology of *Brycon meeki*, a generalized characid fish, with an osteological definition of the family. *Stanford Ichthyological Bulletin*, 8, 1–77.