Ichthyol. Explor. Freshwaters, Vol. 15, No. 3, pp. 243-256, 9 figs., 4 tabs., September 2004
 – ISSN 0936-9902

Three new subterranean species of *Ituglanis* from Central Brazil (Siluriformes: Trichomycteridae)

Maria Elina Bichuette* and Eleonora Trajano**

Three new species (*Ituglanis bambui, I. epikarsticus, I. ramiroi*) are described from different cave systems in São Domingos karst area, State of Goiás, Tocantins River basin, Central Brazil. The three are distinguished from each other and from *I. passensis,* another cave species from that area, by the degree of reduction of eyes and pigmentation, coloration patterns (when present), number of opercular and interopercular odontodes, shape of maxilla, vomer, palatine and urohyal bones, caudal skeleton, in addition to morphometric and meristic characters. Five pairs of ribs were observed in adults *I. epikarsticus* and one cleared and stained juvenile of *I. bambui. Ituglanis ramiroi* showed unique characters: an integument fold anterior and posterior to the dorsal fin and nine pectoral-fin rays. *Ituglanis bambui* inhabits an upper vadose tributary inside Angélica Cave, where it is relatively abundant. *Ituglanis epikarsticus* and *I. ramiroi* are rare in cave habitats, being found respectively in a single set of rimstome pools in São Mateus Cave and in a side pool in São Bernardo Cave. No trichomycterid catfish has been found in epigean streams in the study area. Allopatric models are proposed for the origin of these species based on the local extinction of epigean populations and/or isolation in epikarstic habitats due to alluvial downcutting of the main cave drainages.

Introduction

Brazil has a diverse and abundant hypogean (subterranean) ichthyofauna, with at least 18 troglobitic (exclusively subterranean) species occurring in different karst areas, including those described herein (Trajano, 1997; Trajano & Bichuette, 2003). In this aspect, it compares to Mexico, the Caribbean, China and southeast Asia (Weber et al., 1998).

The Neotropical Trichomycteridae is one of the most diverse siluriform family, comprising at least 35 genera and 200 nominal species (de Pinna, 1998; Wosiacki, 2002) of small-sized catfishes, with cis- and transandine species. The genus *Trichomycterus* Valenciennes, 1833 is the most speciose of the family (over 100 species) and one of the catfish genera best represented in the cave environment, including several species that show reduction of eyes and of melanic pigmentation (troglomorphisms) indicating restriction to subterranean habitats. Up to now, at least seven troglomorphic species were reported: *T. chaberti* from Bolivia; *T. conradi* and *T. spelaeus* from Venezuela; *T. itacarambiensis* from eastern Brazil; and

* Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo. C.P. 11461, 05422-970, São Paulo - SP, Brasil. E-mail: bichuette@uol.com.br

** Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo. C.P. 11461, 05422-970, São Paulo - SP, Brasil. E-mail: etrajano@usp.br

three undescribed species, one from Colombian Andes (Sket, 1988) and two respectively from northeastern and southwestern Brazil (M. de Pinna, pers. comm.).

Costa & Bockmann (1993) erected the genus *Ituglanis* to include nine species previously placed in *Trichomycterus*, based on three autapomorphies (supraoccipital fontanel reduced to a small round orifice, palatine with a deep medial concavity, anterior extremity of sphenotic directed anteriorly). *Ituglanis* species are distributed in cisandine South America and include nine species (Costa & Bockmann, 1993). A recently described cave species, *I. passensis* from Passa Três Cave, in São Domingos karst area, Central Brazil, represents the first record of the genus for the subterranean environment (Fernández & Bichuette, 2002).

The São Domingos region, State of Goiás, is distinguished by its subterranean ichthyofauna particularly rich in both troglomorphic and non-troglomorphic species. This region has the highest diversity of subterranean fishes among the Brazilian karst areas, with seven troglobitic species reported until now (Bichuette & Trajano, 2003; Trajano et al., 2004), including the presently described ones. Fieldwork carried out during the dry seasons of 1999, 2000 and 2001, with collections in subterranean and in epigean stream reaches of São Domingos karst area, yielded three populations of troglobitic trichomycterids other than *I. passensis*. Differences in the external morphology, anatomy, ecology and behaviour were observed among these four populations, justifying the recognition of distinct species. The aim of this paper is to describe the three new cave species of *Ituglanis* from Central Brazil, with habitat and ecological data and evolutionary considerations.

Study site

The three species described herein have been found in caves (respectively, Angélica, São Mateus and São Bernardo caves) situated within the limits of the Terra Ronca State Park (13°30'-13°50'S 46°10'-46°30'W), in São Domingos County, eastern State of Goiás, Central Brazil. This area lies in the Cerrado (the savannah-like Brazilian vegetation) phytogeographic domain (Ab'Saber, 1977), and is characterized by a tropical semi-humid climate with 4-5 dry months per year (Nimer, 1979). São Domingos is a carbonate karst area characterized by the presence of continuous limestone outcrops belonging to the Bambuí Group crossed by several parallel streams running westwards to join the Paranã River, a tributary of the Upper Tocantins River, in the Amazon basin. Characteristically, after an epigean reach, each major stream and some of their tributaries enter into a cave through a sinkhole, cross hundreds to thousands of meters through subterranean conduits, and emerge to the surface through a resurgence. A map showing the rivers and caves from São Domingos Karst area is in Trajano & Bichuette (2003: 1103).

The descriptions of the new species are based on specimens found in cave habitats in the vadose zone (zone of vertical circulation of meteoric water, in opposition to the phreatic, or saturated zone) fed by epikarstic waters. The epikarst is the uppermost layer of a karstified rock (an irregular region with sinks, springs, bare limestone, underground streams and caverns) in which a large proportion of fissures have been enlarged by solutional erosion allowing a rapid infiltration and storage of large quantities of water (Drew, 1995), that may form perched aquifers.

Methods

Fishes were hand-netted, preserved in formalin and transferred to alcohol 70 % for study in laboratory. A small number of specimens were removed from the populations for preservation and behavioral studies in order to avoid important losses for these populations because troglobitic species are intrinsecally fragile, vulnerable to disturbance and easily threatened (Culver, 1986; Trajano, 2001). Moreover, troglobitic *Ituglanis* catfishes are indeed rare in São Mateus and São Bernardo caves. An undetermined species of *Ituglanis* from the Tocantins River basin, in the State of Goiás, representing the closest epigean locality available to us, was used for comparison with the *Ituglanis* populations from caves in the São Domingos karst area.

All measurements were straight-line, taken under stereomicroscope with a dial caliper, 0.1 mm precision, on the left side of specimens. Measurements follow Tchernavin (1944), de Pinna (1992), and Trajano & de Pinna (1996). Counts of dorsal-, anal-fin rays and vertebrae follow de Pinna (1992). Specimens were cleared and double-stained for bone and cartilage by the method

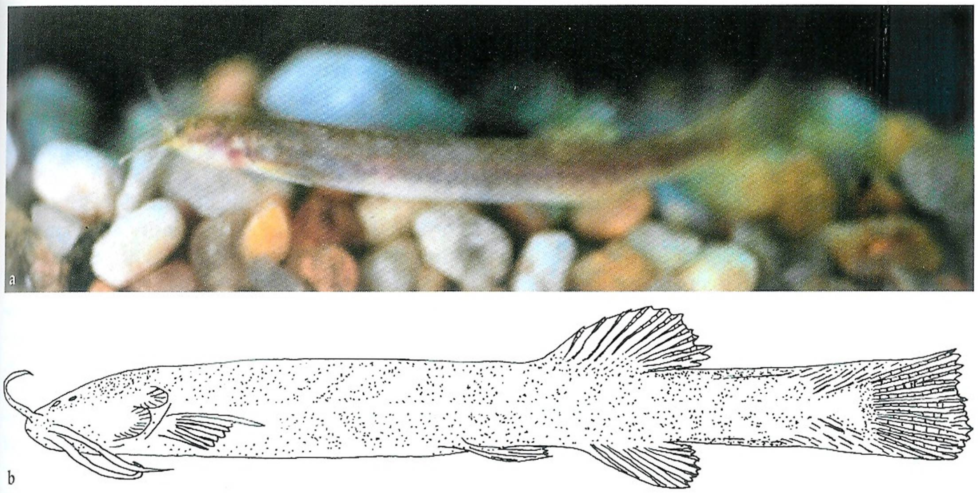


Fig. 1. *Ituglanis bambui*, Brazil: Goiás: Angélica Cave. **a**, not preserved, about 45.0 mm SL; **b**, holotype, MZUSP 79860, 43.2 mm SL.

of Taylor & Van Dyke (1985). Osteological terminology follows de Pinna (1989). Counts of chromatophores were made under stereomicroscope on a 1.0 mm^2 area below the dorsal fin of adults specimens. Counts were also made in the epigean species of *Ituglanis* from the Tocantins river basin. Abbreviations: MZUSP, Museu de Zoologia da Universidade de São Paulo; C&S, cleared and stained; TL, total-length; SL, standard-length; HL, head length.

Minimal population densities were calculated based on visual censuses (Krebs, 1989), taking into account the area of the cave streams and pools inspected for the present study. Spatial distribution in the natural environment was described using the ad libitum method (Lehner, 1996).

Ituglanis bambui, new species
(Fig. 1)

Holotype. MZUSP 79860, 43.2 mm SL; Brazil: Goiás: São Domingos: Terra Ronca State Park: Angélica Cave: upper tributary of main subterranean stream, formed by infiltration of epikarstic water, 13°31'S 46°23'W; M. E. Bichuette, 5 May 2001.

Paratypes. MZUSP 79861, 1, 32.7 mm SL; same locality as holotype; M. E. Bichuette & R. H. Santos, 7 Sep 1999. – MZUSP 79862, 4, 30.6-45.5 mm SL (C&S); same locality as holotype; M. E. Bichuette & R. H. Santos, 3 May 2000. – MZUSP 79863, 3, 35.0-41.5 mm SL (C&S); same locality as holotype; M. E. Bichuette, R. H. Santos & A. Chagas-Jr., 1 Aug 2000. – MZUSP 79864, 4, 31.7-46.3 mm SL (C&S); same locality as holotype; M. E. Bichuette & R. H. Santos, 7 Aug 2001.

Diagnosis. *Ituglanis bambui* is distinguished among its epigean and cave congeners by the combination of the following characters: small size (max. 46.3 mm SL); pigmentation pattern intermediate between epigean and cave *Ituglanis* species, with small light brown spots on body, spots twice larger than in *I. epikarsticus* and *I. ramiroi* (Fig. 1a); eyes relatively developed when compared to cave species but reduced when compared to the epigean ones, with diameter varying from 0.2-0.3 mm (3.4-6.1 % HL) in adults to 0.5 mm (5.7 % HL) in juveniles (vs. 0.8-1.1 mm, 10.4-13.7 % HL in adults of epigean *Ituglanis* species; n=6, 55.7-63.0 mm SL). Maxilla with proeminent medial-posterior projection; fronto-lachrymal as long as maxilla, posteriorly pointed; posterior process of palatine ½ of palatine length, with medial concavity accentuated; vomer elongate with a small constriction in neck; opercle with 11 odontodes and interopercle with 26 odontodes; caudal skeleton with upper hypural plate triangular and lower trapezoidal, neural spine of preural centrum with rounded extremity, dorsal procurrent rays 16 and ventral 14.

Description. Morphometric data of holotype and paratypes are given in Table 1. Body elongate, semi-cylindrical, becoming compressed towards caudal fin. Dorsal and ventral profiles of trunk and caudal peduncle straight (Fig. 1b). Lips and barbels covered by papillae.

Head, depressed, trapezoidal in dorsal view. Eyes reduced in relation to epigean *Ituglanis* species, but visible externally as round black spots covered by thin skin. Anterior nostril transversally ovoid and slightly smaller than posterior nostril, surrounded laterally by nasal barbels. Posterior nostril rounded. Mouth subterminal, rictus posteriorly directed. When adpressed to body, maxillary barbel extending almost to end of pectoral fin, submaxillary barbel to origin of pectoral fin, nasal barbel to middle of pectoral fin.

Pectoral fin triangular in dorsal view, with 8 rays, first ray longer, unbranched and filamentous. Dorsal fin with 10 rays (3 unbranched, 7 branched), semi-circular in lateral view. Anal fin with 9 rays (4 unbranched, 5 branched), semi-circular in lateral view, distal margin slightly rounded. Pelvic fin with 5 rays, first ray unbranched, rectangular in ventral view, splint present. Posterior caudal fin margin straight, with 6+8 principal rays, dorsal procurrent rays 16 and ventral 14.

General morphology of cranium. Main body axis of mesethmoid with similar anterior and posterior widths, width of cornua ¾ of lenght, cornua with acute distal extremity; posttemporo-supracleithrum not elongate, with dorsal limb lying in pterotic. Three premaxillary tooth rows. Maxilla with proeminent medial-posterior projection. Fronto-lachrymal as long as maxilla, posteriorly pointed (Fig. 2). Branchiostegal rays six. Base of laminar surface of urohyal four times larger than distal extremity; urohyal dorsal process short (Fig. 3a). Length of posterior process of palatine ½ of palatine length, medial concavity accentuated (Fig. 3b). Vomer elongate, neck with small constriction. Opercle with 11 odontodes and interopercle with 26 odontodes.

Postcranial skeleton. Total vertebrae 36-37; six pairs of ribs. Epural absent. Neural spine (n=1) of preural centrum with rounded extremity. Upper hypural plate triangular, lower hypural plate trapezoidal. Uroneural with acute distal extremity (Fig. 3c).

Table 1. Morphometric data of holotype (H) and 12 paratypes of *Ituglanis bambui*.

										H			
Standard length (mm)	30.6	31.7	31.9	32.0	32.7	35.0	39.0	40.8	41.5	43.2	43.6	45.5	46.3
Total length (mm)	34.9	36.6	36.6	37.3	38.1	39.5	45.0	47.1	46.8	47.6	51.0	52.3	52.9
Percentages of standard length													
Body depth	10.0	9.7	11.0	10.0	9.2	10.0	9.0	10.5	10.0	9.1	9.0	10.7	10.0
Caudal peduncle length	18.0	14.5	18.0	17.0	17.4	15.0	19.4	18.6	16.0	14.8	18.0	14.9	16.0
Caudal peduncle depth	7.0	5.0	8.0	7.0	6.7	7.0	7.7	9.1	10.0	5.1	7.0	9.2	9.0
Predorsal length	66.0	69.7	67.0	67.0	56.2	64.0	67.0	72.5	68.0	66.7	69.0	70.3	68.0
Preanal length	68.0	71.9	71.0	72.0	59.3	67.0	69.4	69.8	73.0	69.2	72.0	78.0	72.0
Prepelvic length	56.0	59.6	54.0	50.0	47.4	56.0	57.0	58.1	59.0	55.1	59.0	59.3	60.0
Dorsal-fin base length	10.0	6.9	10.0	8.0	7.9	7.0	6.9	7.8	9.0	7.9	8.0	9.6	8.0
Anal-fin base length	9.0	8.2	8.0	9.0	7.6	6.0	6.7	7.4	11.0	7.9	7.0	8.6	6.0
Head length	17.0	17.0	16.0	16.0	15.0	17.0	16.7	16.2	17.0	16.2	16.0	15.2	17.0
Head width	14.4	17.4	16.3	16.3	16.5	16.6	16.4	17.2	16.6	14.3	16.3	16.9	17.1
Head depth	8.5	9.1	9.4	9.1	7.3	8.6	8.2	8.1	8.9	6.5	8.0	7.9	8.9
Percentages of head length													
Nasal barbel length	90	66	90	67	77	91	106	69	84	81	65	75	83
Maxillary barbel length	116	98	108	100	100	107	120	92	103	84	91	107	84
Submaxillary barbel length	78	61	79	58	63	78	75	59	80	70	64	51	68
Mouth width	53	61	56	54	51	53	52	68	61	54	59	68	60
Internarial width	19	20	25	25	22	20	21	19	24	22	20	20	22
Interorbital	31	27	32	36	32	34	30	33	35	30	29	33	36

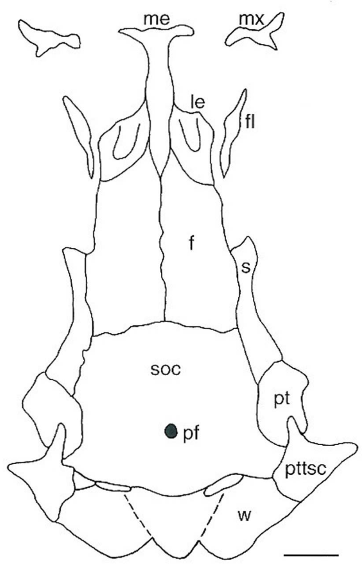


Fig 2. *Ituglanis bambui*. Schematic dorsal view of neurocranium and maxillae. Abbreviations: **f**, frontal; **fl**, fronto-lachrymal; **le**, lateral ethmoid; **me**, mesethmoid; **mx**, maxilla; **pf**, posterior fontanel; **pt**, pterotic; **pttsc**, posttemporosupracleithrum; **s**, sphenotic; **soc**, supraoccipital; **w**, weberian complex. Scale bar 1.0 mm.

Color of living specimens. Irregular light brown spots along dorsum and flanks (Fig. 1a). Eye black. Dorsal portion of head darker than the rest of body, mainly between eyes. Juveniles less pigmented than adults, dorsal region of skull darker than in other parts of body.

Color in alcohol. Pale light brown in adults and pale yellowish in juveniles. Eye black. Fins poorly pigmented, translucent. Dorsal portion of head light gray. Density of chromatophores ranging from 6.0 to 19.5 per mm^2 (n=7, mean=9.8, sd=4.69), chromatophores coloration varying from light brown to black points.

Juvenile. One cleared and stained juvenile of *I. bambui* (21.5 mm SL, non type) differs from the adults by: posterior fontanel wide and directed anteriorly, like a window; seven branchiostegal rays; five pairs of ribs.

Ecology and behaviour. *Ituglanis bambui* inhabits an upper vadose tributary of Angélica stream inside Angélica Cave, near the sinkhole end (where the epigean stream sinks into the cave).

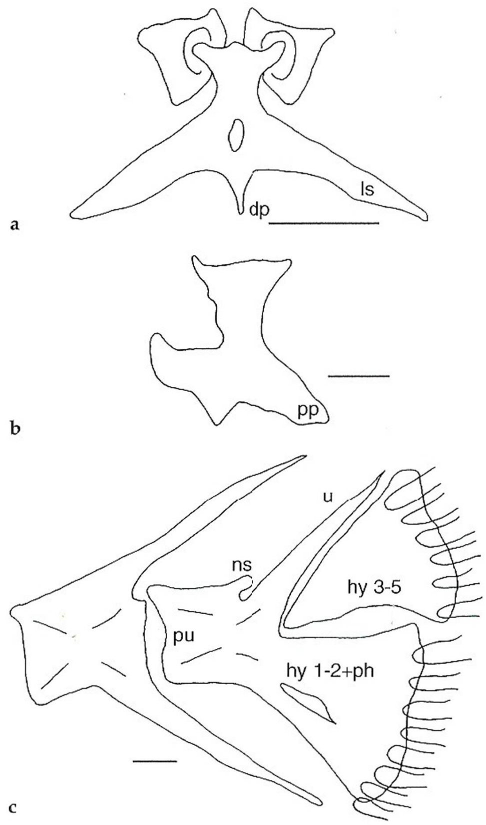


Fig. 3. *Ituglanis bambui*. **a**, ventral view of urohyal; **b**, left palatine; **c**, left lateral view of caudal skeleton. Abbreviations: **dp**, dorsal process; **hy**, hypural; **ls**, laminar surface; **ns**, neural spine; **ph**, parahypural; **pp**, posterior process; **pu**, preural centrum; **u**, uroneural. Scale bars 1.0 mm.

This tributary is characterized by slow-moving waters, 10-150 cm deep on average, and bottom formed basically by silt and clay, with some rocky blocks. Based on visual censuses and on the area of the accessible fish habitat (approximately 250 m long and 4.5 m wide in average), minimum population density in the studied site was estimated around 0.04 indiduals per m^2. Environmental variables measured in May, July and August 2000 (dry season): water temperature 23.6, 19.9 and 21.6 °C; pH 7.9, 8.6, and 8.0; dissolved oxygen 8.2, 7.7 and 6.6 mg · l^{-1}. The pH values are typical of a subterranean karst drainage (Culver, 1982). In all occasions the individuals were observed solitary, with swimming activity on the bottom, in midwater and sometimes extending to the surface. No observed fish displayed cryptobiotic habits, never trying to hide or bury into the soft bottom when disturbed. Specimens of *Ituglanis*, probably also *I. bambui*, have been observed (but not collected) in a second upper tributary situated at the opposite end of the cave, near the stream resurgence

Distribution. *Ituglanis bambui* is known from two upper tributaries of the main stream in the Angélica Cave which are 7,000 m far apart, indicating a wide distribution in the epikarst.

Etymology. The specific name makes reference to the carbonate geological unit, the Bambuí Group, where it occurs, and it is also a recognition to the Brazilian speleological association, Grupo Bambuí de Pesquisas Espeleológicas (GBPE), for its contribution to Brazilian speleology.

Ituglanis epikarsticus, new species
(Fig. 4)

Holotype. MZUSP 79869, 26.0 mm SL; Brazil: Goiás: São Domingos: Terra Ronca State Park: São Mateus Cave: rimstone dams (travertine basins) fed by infiltration of epikarstic water (13°40'S 46°22'W); M. E. Bichuette, 11 Aug 2001.

Paratypes. MZUSP 79870, 1, 34.0 mm SL; same locality as holotype; M. E. Bichuette & F. C. T. Lima, 15 July 1999. – MZUSP 79871, 2, 29.2 and 30.6 mm SL (C&S); same locality as holotype; M. E. Bichuette, 24 May 1999. – MZUSP 79872, 1, 29.9 mm SL (C&S); same locality as holotype; M. E. Bichuette & F. D. Passos, 11 Aug 2001.

Diagnosis. *Ituglanis epikarsticus* is distinguished from its epigean and cave congeners by the combination of the following characters: small size (max. 34.0 mm SL); the higher degree of reduction in body pigmentation and in eyes size, 2.0-2.1 % HL (Figs. 4a-b); supraoccipital fontanel very reduced or absent; base and distal extremity of laminar surface of urohyal with similar widths, dorsal process short; posterior process of palatine as long as palatine length, medial concavity slightly rounded; maxilla with discrete medial-posterior projection; fronto-lachrymal ⅔ length of maxilla, anteriorly and posteriorly pointed; opercle with 9 odontodes and interopercle with 20-21 odontodes; 5 pairs of ribs (also observed in a juvenile of *I. bambui*); caudal skeleton with upper hypural plate triangular and lower one rectangular, neural spine of preural centrum with acute extremity, dorsal procurrent rays 14 and ventral 10.

Description. Morphometric data of holotype and paratypes are given in Table 2. Body elongate, semi-cylindrical, becoming compressed towards caudal fin. Dorsal and ventral profiles of trunk and caudal peduncle straight (Fig. 4b). Lips and barbels covered by papillae.

Head trapezoidal in dorsal view. Eyes appearing as very small round black spots covered by thin skin. Eyes diameter around 0.1 mm in two individuals. Anterior nostril rounded, surrounded laterally by nasal barbels. Posterior nostril semi-circular. Mouth subterminal, with rictus slightly straight. Maxillary barbel extending to origin of pectoral fin, submaxillary barbel to end of interopercle, nasal barbel to end of interopercle.

Pectoral fin triangular in dorsal view, with 8 rays, first ray longer, unbranched and filamentous. Dorsal fin with 11 rays (3 unbranched, 8 branched), trapezoidal in lateral view. Anal fin

Table 2. Morphometric data of holotype (H) and 4 paratypes of *Ituglanis epikarsticus*.

	H				
Standard length (mm)	26.0	29.2	29.9	30.6	34.0
Total length (mm)	30.0	34.0	35.2	35.4	38.6
Percentages of standard length					
Body depth	8.5	7.9	7.7	7.5	9.4
Caudal peduncle length	17.0	12.7	15.1	10.5	18.5
Caudal peduncle depth	5.4	7.2	4.7	6.2	4.4
Predorsal length	65.0	72.6	67.2	69.3	71.2
Preanal length	70.4	73.6	74.2	76.5	70.3
Prepelvic length	54.6	58.2	59.3	57.6	55.9
Dorsal-fin base length	10.0	11.6	8.4	8.8	10.6
Anal-fin base length	11.2	6.8	9.7	7.2	8.5
Head length	15.4	16.1	17.7	16.3	16.5
Head width	17.3	17.5	15.1	17.6	13.2
Head depth	9.6	7.9	7.0	8.2	7.4
Percentages of head length					
Nasal barbel length	82	55	58	60	62
Maxillary barbel length	110	115	102	104	106
Submaxillary barbel length	72	51	56	54	59
Mouth width	61	53	52	50	49
Internarial width	20	21	22	22	14
Interorbital	30	25	22	26	26

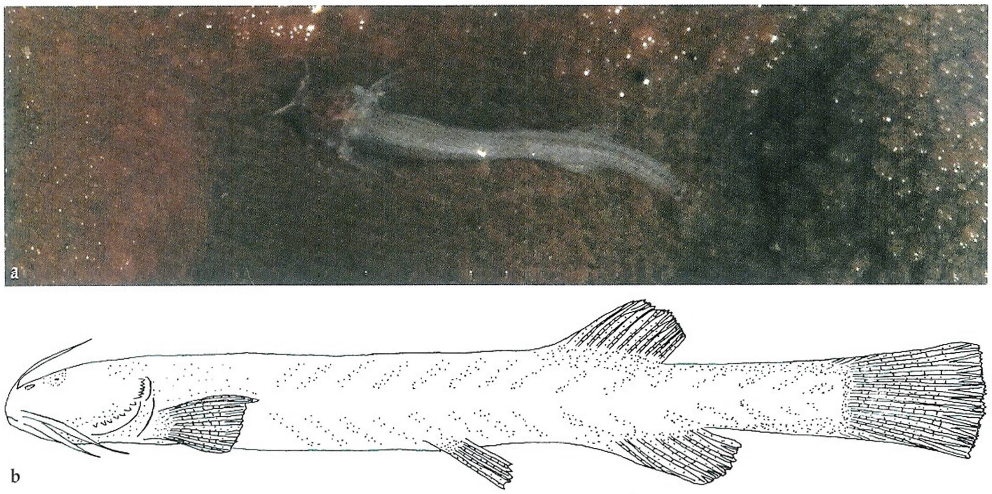


Fig. 4. *Ituglanis epikarsticus*, Brazil: Goiás: São Mateus Cave. **a,** not preserved, about 29.0 mm SL, in natural habitat; **b,** holotype, MZUSP 79869, 26.0 mm SL.

with 8 rays (2 unbranched, 6 branched), trapezoidal in lateral view. Pelvic fin with 5 rays, first ray unbranched, rectangular in ventral view, splint present. Posterior caudal fin margin straight, truncate in dorsal and ventral edges, with 6+8 principal rays, dorsal procurrent rays 14 and ventral 10.

General morphology of cranium. Main body axis of mesethmoid with anterior and posterior portions with same width, base of cornua ⅔ of length, distal extremity acute; posttemporosupracleithrum elongate with dorsal limb lying in pterotic. Three premaxillary tooth rows. Maxilla with discrete medial-posterior projection. Fronto-lachrymal ⅔ length of maxilla, anteriorly and posteriorly pointed (Fig. 5). Base and distal extremity of laminar surface of urohyal with similar widths, dorsal process of urohyal short (Fig. 6a). Posterior process of palatine as long as palatine length, medial concavity slightly rounded (Fig. 6b). Vomer short when compared to *I. bambui*. Opercle with 9 odontodes, interopercle with 20-21 odontodes.

Postcranial skeleton. Total vertebrae 36; five pairs of ribs. Epural absent. Neural spine (n=1) of preural centrum with acute extremity. Upper hypural plate triangular, lower hypural plate rectangular. Uroneural straight, with rounded extremity (Fig. 6c).

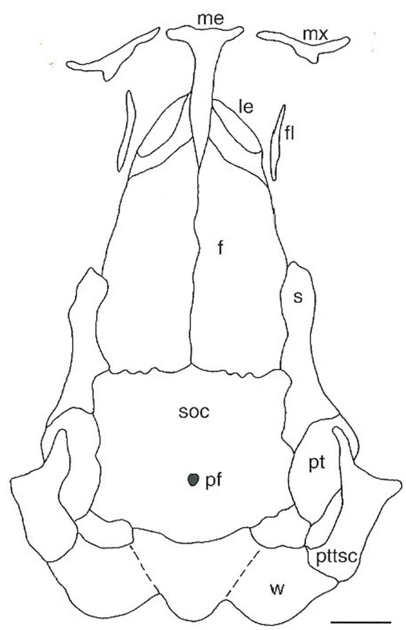


Fig. 5. *Ituglanis epikarsticus*. Schematic dorsal view of neurocranium and maxilla. Abbreviations: **f**, frontal; **fl**, fronto-lachrymal; **le**, lateral ethmoid; **me**, mesethmoid; **mx**, maxilla; **pf**, posterior fontanel; **pt**, pterotic; **pttsc**, posttemporosupracleithrum; **s**, sphenotic; **soc**, supraoccipital; **w**, weberian complex. Scale bar 1.0 mm.

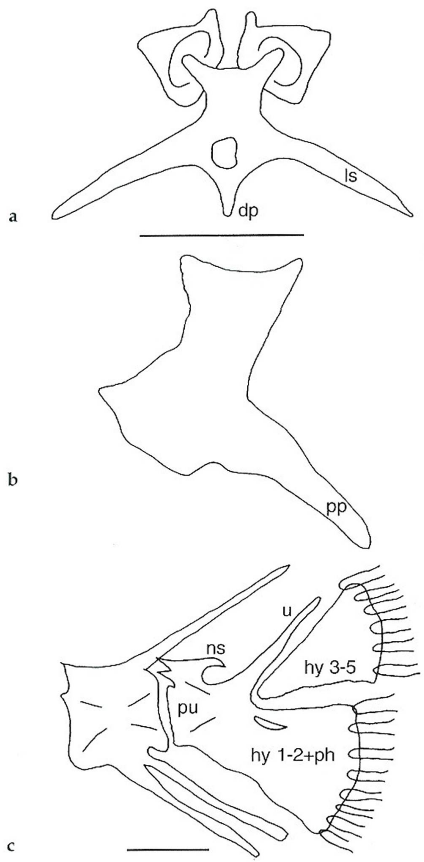


Fig. 6. *Ituglanis epikarsticus*. **a,** ventral view of urohyal; **b,** left palatine; **c,** left lateral view of the caudal skeleton. Abbreviations: **dp,** dorsal process; **hy,** hypural; **ls,** laminar surface; **ns,** neural spine; **ph,** parahypural; **pp,** posterior process; **pu,** preural centrum; **u,** uroneural. Scale bars 1.0 mm.

Color of living specimens. Pinkish-white, transparent in one individual. Dorsal region of skull darker than other parts of body. Eye spot black. Fins not transparent (Fig. 4a).

Color in alcohol. Pale yellowish with very few scattered chromatophores (very small black points) distributed along dorsum and flanks of body. Density of chromatophores 6.0 to 7.5 per mm^2 (n=2).

Ecology and behaviour. *Ituglanis epikarsticus* has been found in pools in a set of rimstone dams (wall-shaped barriers of calcite, aragonite or other chemical deposits that impound water pools), 10-30 cm deep and with rocky bottom.These pools are fed by epikarst waters percolating from the ceiling. Density of fish in the rimstone pools (about 20 m^2) was estimated around 0.08 individuals per m^2. Environmental variables measured in May 1999 (dry season) in the rimstone pools: water temperature 24.8 °C; pH 7.7 and dissolved oxygen 7.7 $mg \cdot l^{-1}$. Different values were obtained in this occasion at the main cave stream: temperature 21.9 °C; dissolved oxygen 9.3 $mg \cdot l^{-1}$. The rare fishes seen were solitary, swimming on the bottom, along the walls and sometimes at the surface of the pools. Like *I. bambui*, and contrasting with *I. passensis*, *I. epikarsticus* did not exhibit any cryptobiotic habits.

Distribution. *Ituglanis epikarsticus* is known from a single set of rimstone dams in São Mateus Cave.

Etymology. The specific name, *epikarsticus*, makes reference to the type of aquifer where the species occurs. An adjective.

Ituglanis ramiroi, new species
(Fig. 7)

Holotype. MZUSP 79865, 27.1 mm SL; Brazil: Goiás: São Domingos: Terra Ronca State Park: São Bernardo Cave: side pool fed by small water inlet (13°49'S 46°21'W), for about 700 m from cave entrance; M. E. Bichuette, 12 Aug 1999.

Paratypes. MZUSP 79866, 2, 30.2-30.9 mm SL (C&S); same locality as holotype; M. E. Bichuette & R. H. Santos, 21 May 1999. – MZUSP 79867, 3, 25.4-31.3 mm SL (C&S); same locality as holotype; M. E. Bichuette & R. H. Santos, 27 May 1999. – MZUSP 79868, 1, 29.3 mm SL; same locality as holotype; M. E. Bichuette & R. H. Santos, 12 July 1999.

Diagnosis. *Ituglanis ramiroi* is distinguished from its epigean and cave congeners by the combination of the following characters: small size (max. 31.3 mm SL); reduced body pigmentation, with very small chromatophores, dorsal region of head darker than remaining body parts; small integument fold anterior to dorsal fin and proeminent posterior to dorsal fin (Fig. 7a); eye size intermediate between *I. bambui* and *I. epikarsticus*, 3.7-4.7 % HL; nine pectoral-fin rays; base of laminar surface of urohyal 1.5 times larger than distal extremity, dorsal process long; posterior process of palatine ¾ of palatine length, with medial concavity slightly rounded; maxilla straight, without medial-posterior projection; fronto-lachrymal as long as maxilla, posteriorly pointed; opercle with 12-13 odontodes, interopercle with 24-25 odontodes; caudal skeleton with the upper hypural plate trapezoidal and the lower trapezoidal to rectangular, neural spine of preural centrum with acute extremity, dorsal procurrent rays 16 and ventral 12.

Description. Morphometric data of holotype and paratypes are given in Table 3. Body elongate, cylindrical, becoming compressed towards caudal fin. Dorsal and ventral profiles of trunk and caudal peduncle dislodged, with the caudal peduncle not broad like the trunk (Fig. 7b). Lips and barbels covered by papillae.

Head trapezoidal in dorsal view. Eyes appear as very small round black spots covered by thin skin. Eyes diameter 0.2 mm in three individuals. One paratype with eyes not externally visible. Anterior nostril rounded, surrounded laterally by nasal barbels. Posterior nostril semi-circular. Mouth subterminal, rictus accentuatedly directed posteriorly. Maxillary barbel extending to origin of pectoral-fin; submaxillary barbel extending to end of opercle; nasal barbel extending to end of interopercle.

Pectoral fin triangular in dorsal view, with 9 rays, first ray unbranched and filamentous. Dorsal fin with 9 rays (3 unbranched, 6 branched), trapezoidal in lateral view. Anal fin with 6 rays (2 unbranched, 4 branched), rectangular in lateral view. Pelvic fin with 5 rays, first ray unbranched, triangular in ventral view, splint present. Posterior caudal fin margin straight, slightly rounded in dorsal and ventral edges, with 6+7 principal rays, dorsal procurrent rays 16 and ventral 12.

General morphology of cranium. anterior portion of main body of mesethmoid ⅔ of posterior portion, basis of cornua approximately ½ of length, with rounded distal extremity; posttemporosupracleithrum elongate with a short dorsal limb lying in pterotic. Three premaxillary tooth rows. Maxilla without medial-posterior projection, straight. Fronto-lachrymal as long as maxilla, posteriorly pointed (Fig. 8). Base of laminar surface of urohyal 1.5 times larger than distal extremity, dorsal process of urohyal long (Fig. 9a); posterior process of palatine ¾ of palatine length,

Table 3. Morphometric data of holotype (H) and 6 paratypes of *Ituglanis ramiroi*.

		H					
Standard length (mm)	25.4	27.1	29.3	30.2	30.5	30.9	31.3
Total length (mm)	30.3	31.9	33.5	34.4	34.1	36.0	36.4
Percentages of standard length							
Body depth	10.0	14.0	14.0	10.0	12.0	11.0	13.0
Caudal peduncle length	15.0	17.0	16.0	17.0	15.0	10.0	16.0
Caudal peduncle depth	6.0	6.0	4.8	9.0	6.0	8.0	6.0
Predorsal length	66.0	69.0	66.0	71.0	66.0	72.0	67.0
Preanal length	71.0	70.0	69.0	73.0	68.0	75.0	69.0
Prepelvic length	58.0	58.0	56.0	56.2	53.0	57.5	56.0
Dorsal-fin base length	9.0	11.0	11.0	9.0	9.0	8.7	10.0
Anal-fin base length	11.0	8.0	9.0	8.0	9.0	8.1	10.0
Head length	14.0	15.0	15.0	17.0	16.0	17.0	17.0
Head width	16.1	18.1	16.0	17.9	14.8	16.5	18.2
Head depth	9.0	11.0	10.2	9.0	9.5	10.0	8.0
Percentages of head length							
Nasal barbel length	86	85	88	81	84	81	79
Maxillary barbel length	125	120	123	98	99	108	102
Submaxillary barbel length	67	63	74	54	56	50	62
Mouth width	57	51	51	46	45	51	51
Internarial width	25	24	25	19	20	19	22
Interorbital	31	31	32	25	30	27	28

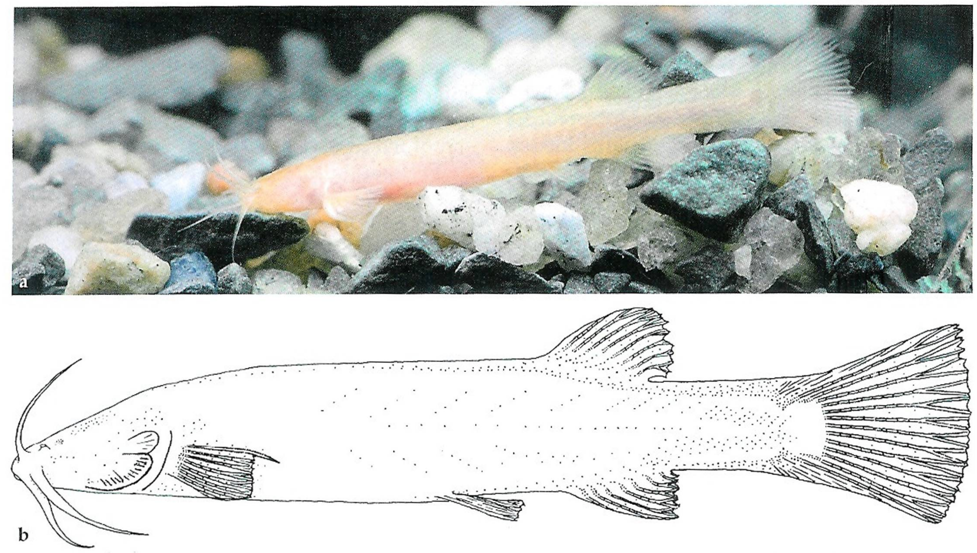


Fig. 7. *Ituglanis ramiroi*, Brazil: Goiás: São Bernardo Cave. **a**, not preserved, about 33.0 mm SL; **b**, holotype, MZUSP 79865, 27.1 mm SL.

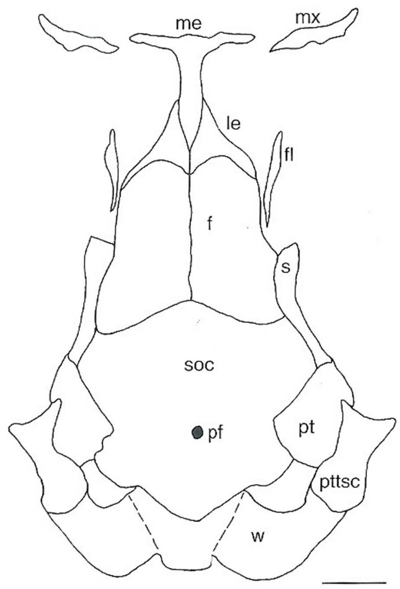


Fig. 8. *Ituglanis ramiroi*. Schematic dorsal view of neurocranium and maxilla. Abbreviations: **f**, frontal; **fl**, fronto-lachrymal; **le**, lateral ethmoid; **me**, mesethmoid; **mx**, maxilla; **pf**, posterior fontanel; **pt**, pterotic; **pttsc**, posttemporosupracleithrum; **s**, sphenotic; **soc**, supraoccipital; **w**, weberian complex. Scale bar 1.0 mm.

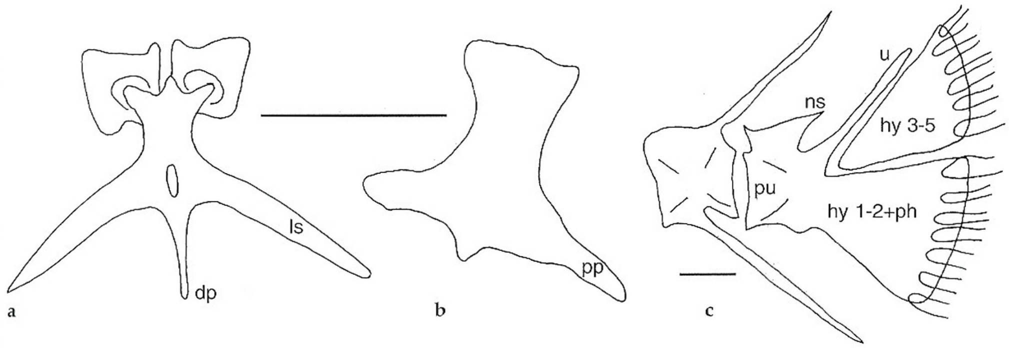
medial concavity slightly rounded (Fig. 9b); vomer shorter than in *I. bambui*, with an enlargement in medial region; opercle with 12-13 odontodes, interopercle with 24-25 odontodes.

Postcranial skeleton. Total vertebrae 36; six pairs of ribs. Epural absent. Neural spine (n=1) of preural centrum with acute extremity. Upper hypural plate trapezoidal, lower hypural plate trapezoidal to rectangular. Uroneural straight, with acute extremity (Fig. 9c).

Color of living specimens. Pale yellowish with scattered chromatophore spots along dorsum and flanks of body. Eye spots black. Fins without pigments, with translucent aspect (Fig. 5a).

Color in alcohol. Pale yellowish to white. Eye spots black. Density of chromatophores ranging from 9.0 to 12.0 per mm^2 (n=3), chromatophores coloration varying from light brown to very small reddish points.

Ecology and behaviour. *Ituglanis ramiroi* has been found in a side pool five meters above the main stream level, formed by water percolating from the rock. The water current in the pool was slow, depth varied from 10 to 40 cm and the bottom was formed basically by silt and gravel, with



a b c

Fig. 9. *Ituglanis ramiroi*. **a**, ventral view of urohyal; **b**, left palatine; **c**, left lateral view of the caudal skeleton. Abbreviations: **dp**, dorsal process; **hy**, hypural; **ls**, laminar surface; **ns**, neural spine; **ph**, parahypural; **pp**, posterior process; **pu**, preural centrum; **u**, uroneural. Scale bars 1.0 mm.

some boulders. Density of fish in the pool (around 18 m × 1.3 m) was estimated around 0.3 individuals per m². Environmental variables measured in May 1999, July 2000 and August 2001 (dry seasons): water temperature 23.1, 23.0 and 22.5 °C; pH 6.76, 7.5 and 7.3; and dissolved oxygen 5.1, 4.7 and 5.4 mg · l⁻¹. The relatively low values of dissolved oxygen are probably related to the slow movement of water in the pool. In all occasions, the individuals were observed solitary, swimming on the bottom and displaying rare events of surface swimming. *Ituglanis ramiroi* showed weak cryptobiotic habits, sometimes hiding under boulders, but not burying in the silt.

Distribution. *Ituglanis ramiroi* is known only from a pool formed by infiltration of water in the São Bernardo Cave (13°49'S 46°21'W).

Etymology. The specific name honours Ramiro Hilário dos Santos, local inhabitant and guide in Terra Ronca State Park, who discovered these catfishes and an enthusiastic supporter of the protection of caves in the area.

Discussion

According to Costa & Bockmann (1993), *Ituglanis* is defined by three autapomorphies: supraoccipital fontanel reduced to a small round orifice, palatine with a deep medial concavity and anterior extremity of the sphenotic directed anteriorly; all of them are present in the three species herein described.

Ituglanis bambui, *I. epikarsticus* and *I. ramiroi* may be distinguished from the epigean *Ituglanis* sp. from Tocantins river basin mainly by the reduction of eyes, the lower density of chromatophores (respectively, 6.0-19.5, 6.0-7.5 and 9.0-12.0 per mm², vs. 58.0-119.0 in six specimens of epigean *Ituglanis* sp., MZUSP 53222), and the smaller body sizes (see diagnoses). In general, epigean species of *Ituglanis* have dark chromatophores, with coloration patterns ranging from chocolate brown, with few darker spots, to dark brown with numerous spots on the body (Eigenmann, 1918; Costa & Bockmann, 1993; Fernández, 2001). The troglobitic status of the three new species is further supported by the fact that no specimen of *Ituglanis* has been so far found in the epigean streams in the area, in spite of an intensive collecting effort. Therefore, the troglobitic species of *Ituglanis*, including *I. passensis*, from São Domingos karst area, may represent geographic or distributional relicts (sensu Holsinger, 1988).

Table 4 summarizes the differences between the three new species and *I. passensis*, showing the mosaic distribution of characters among these species. Althought the degree of body pigmentation and eyes sizes are the most evident diagnostic characters for the subterranean species of *Ituglanis*, the combination of morphometric and osteological characters are also diagnostic.

Several authors advocate a correlation between time of isolation in the subterranean habitat and degree of reduction of eyes and of pigmentation, with the populations showing less reduced and/or variable eyes and pigmentation isolated for shorter times than those homogeneously anophthalmic and unpigmented (Wilkens, 1982, 1986; Trajano, 1995). From this, it may be concluded that *I. bambui*, which presents a lower degree of regression of body pigmentation and eyes, has been isolated in the subterranean environment for a shorter time than *I. passensis*, *I. ramiroi* and *I. epikarsticus*. However, *I. bambui* exhibited the most reduced cryptobiotic habits and an important midwater and surface activity, which are considered apomorphic, advanced traits for troglobitic catfishes (Trajano & Bockmann, 1999).

The larger size of *I. bambui*, intermediate in relation to *I. passensis* (that lives in a base-level cave stream), may be related to life in more spacious cave conduits, supplied by water from the epikarst, when compared to the putative habitats of *I. ramiroi* and *I. epikarsticus*, probably largely restricted to small epikarstic spaces. We hypothesize an evolutionary decrease in body size in the latter (small size as apomorphic condition), pre- or post-colonization of subterranean non-cave habitats.

There are evidence that the four troglobitic *Ituglanis* species from São Domingos region originated independently from one or more epigean ancestors, based not only on differences on morphological character states, which show a mosaic distribution in these species, but also on geological data. Subterranean dispersion by troglobitic fish between these caves seems to be highly improbable, precluding population interchanges. To test this hypothesis, a phylogenetic analysis is necessary.

The vadose tributary, the rimstone pools and the pools fed by epikarstic waters where *I. bambui*, *I. epikarsticus* and *I. ramiroi* have been respectively found are nowadays mostly isolated from

Table 4. Comparative data of subterranean species of *Ituglanis* from São Domingos karst area, Central Brazil. Data for *I. passensis* from Fernández & Bichuette (2002) and preserved specimens.

	I. passensis	*I. bambui*	*I. epikarsticus*	*I. ramiroi*
Maximum SL	62.6 mm	46.3 mm	34.0 mm	31.3 mm
Body pigmentation	yellowish to light gray	pale light brown	pale yellowish	pale yellowish to white
Chromatophores per mm^2	8.0-11.0	6.0-19.5	6.0-7.5	9.0-12.0
Eye diameter (% of HL)	0.4-0.5 mm (3.9-5.2)	0.2-0.3 mm (3.4-6.1)	0.1 mm (2.0-2.1)	0.2 mm (3.7-4.7)
Odontodes [opercular/interopercular]	16/24	11/26	9/20-21	12-13/24-25
Pectoral fin rays	8	8	8	9
Maxilla shape	no medial-posterior projection	proeminent medial-posterior projection	discrete medial-posterior projection	no medial-posterior projection
Fronto-lacrhrymal bone	½ length of maxilla, posteriorly pointed	½ length of maxilla, posteriorly pointed	⅔ length of maxilla, anteriorly and posteriorly pointed	equal to length of maxilla, posteriorly pointed
Vomer shape	elongate, enlargement in medial region	elongate, constriction in neck	short, no constriction	short, enlargement in medial region
Palatine shape	medial concavity rounded	medial concavity accentuated	medial concavity rounded	medial concavity rounded
Caudal skeleton (extremity of neural spine of preural centrum)	acute	rounded	acute	acute
Caudal skeleton (dorsal and ventral procurrent rays)	15 and 11	16 and 14	14 and 10	16 and 12
pairs of ribs	7	6 (one juvenile: 5)	5	6

the main, base-level drainage by differences in topographic level, as a consequence of alluvial downcutting resulting in a regional lowering of the water table. Epigean *Ituglanis* ancestors probably lived in water courses at the preterite base level, directly flowing into caves and allowing for the establishment of cave populations. As a consequence of alluvial downcutting, such formerly running-water streams would become perched and fed by infiltration water from the epikarst. Therefore, differences in topographic level may account for (at least partial) isolation of fish populations surviving in perched water bodies. On the other hand, there is evidence of local extinction of epigean relatives due to some unknown factor. At this point, it is not possible to know which (if any) isolation event, extinction of epigean populations or spatial, topographic isolation, represents the main speciation factor for the troglobitic *Ituglanis*.

Conservation remarks. Troglobitic species are fragile, highly vulnerable to environmental disturbance, due to their generally high degree of endemism, low population sizes, high sensitivity to stressors, and a K-selected life style leading to slow population turnover (Trajano, 2000). As a matter of fact, many hypogean fishes are considered as endangered by different types of threats (Proudlove, 2001)

The three type-localities of the presently described *Ituglanis* species are within the boundaries of a State Park, the Parque Estadual de Terra Ronca. Nevertheless, control of visitation in caves, the only threat currently identifiable, is poorly enforced and does not guarantee full protection for these (and other) species.

Ituglanis bambui has a relatively large population (MEB unpubl.), and the vadose tributary where it occurs is outside the main visitation pathway in Angélica Cave. Therefore, unless visitation greatly increases, this population seems to be relatively safe. In contrast, *I. epikarsticus* is extremely rare in the cave habitat (there is no data on the situation in non-cave, epikarstic habitat), and the number of observed specimens conspicuously decreased since the 1970's (ET, pers. obs.). So far, it has only been found in a set of rimstone pools situated right in the pathway for visitors reaching the far end of the cave. Thus, there is reason for major concern about the future of this endemic species. A strict control of visitation is urgently needed to assure its protection, until evidence appears that the species is safe in non-cave habitats. The situation is less clear for *I. ramiroi*. It is rare in the cave habitat, but the side pool where it was found is easily avoided by visitors, once properly advised by the guides.

Comparative material. *Ituglanis* sp.: MZUSP 53222, 6; Brazil: Goiás: Minaçú: tributary of Rio Tocantinzinho. *I. passensis*: MZUSP 80097, 3, Brazil: Goiás: São Domingos, Passa Três Cave. *Trichomycterus* sp.: MZUSP 60205, 10, Brazil: São Paulo: Barra do Turvo, Ribeirão Fria.

Acknowledgements

We are greatly indebted to the several colleagues and cavers who helped us in the field, especially to Lília S.Horta, who gave us the first specimens from Angélica Cave, and to Ramiro H. Santos, a very helpful and friendly guide, who brought *I. ramiroi* into our attention. Thanks are also due to the Agência Ambiental de Goiás, for permission to develop the study in Terra Ronca State Park (permission # 028/98), and especially to the Park manager, Regina B. Schulz and to the Park driver Arnor V. Mello, for allowing the use of the Park facilities. We are grateful to Osvaldo Oyakawa (MZUSP) for the loan of comparative material, to João Allievi, for allowing us use of figure 4a, and to the Departamento de Zoologia-IBUSP, for support to develop this study. The drawings were prepared by Ricardo C. Rosas, Maria Elina Bichuette and Flávio D. Passos. IBAMA granted us permission for collections (license # 02001.005646/98-40). Financial support was given by FAPESP (scholarship # 98/13858-1). The junior author is partially supported by the CNPq (fellowship # 306066/88-2).

Literature cited

Ab'Saber, A. N. 1977. Os domínios morfoclimáticos na América do Sul. Geomorfologia, 52: 1-21.

Bichuette, M. E. & E. Trajano. 2003. Epigean and subterranean ichthyofauna from São Domingos karst area, Upper Tocantins river basin, Central Brazil. J. Fish Biol, 63: 1100-1121.

Costa, W J. E. M. & F. A. Bockmann. 1993. Un nouveau genre néotropical de la famille des Trichomycteridae (Siluriformes: Loricarioidei). Rev. Fr. Aquariol., 20: 43-46.

Culver, D. C. 1982. Cave life, evolution and ecology. Harvard University Press, Cambridge, 189 pp.

— 1986. Cave faunas. Pp. 427-443 in: M. E. Soulé (ed.), Conservation biology - the science of scarcity and diversity. Sinauer, Sunderlands.

Drew, D. 1995. Glossary of karstic terminology. In: COST Action 65 report "Hydrogeological aspects of groundwater protection in karstic areas". European Commission, Luxembourg, 446 pp.

Eigenmann, C. H. 1918. The Pygidiidae, a family of South American catfishes. Mem. Carnegie Mus., 7: 259-398.

Fernández, L. 2001. Nuevos registros de *Trichomycterus* Valenciennes e *Ituglanis* Costa y Bockmann (Teleostei: Siluriformes) em Argentina, Paraguay y Bolívia. Neotropica, 47: 103-105.

Fernández, L. & M. E. Bichuette. 2002. A new cave dwelling species of *Ituglanis* from the São Domingos karst, central Brazil (Siluriformes: Trichomycteridae). Ichthyol. Explor. Freshwaters, 13: 273-278.

Holsinger, J. R. 1988. Troglobites: the evolution of cave-dwelling organisms. Amer. Sci., 76: 146-153

Karmann, I., L. E. Sánchez & P. Milko. 1984. Proposta preliminar de uma unidade de conservação para as cavernas de São Domingos, Goiás. Espeleo-Tema, 14: 36-42.

Krebs, C. J. 1989. Ecological methodology. Harper Collins, New York, 654 pp.

Lehner, P. N. 1996. Handbook of ethological methods. Cambridge University Press, Cambridge, 672 pp.

Nimer, E. 1979. Climatologia do Brasil. SUPREN, Rio de Janeiro, Brasil, 421 pp.

de Pinna, M. C. C. 1989. A new sarcoglanidine catfish, phylogeny of its subfamily, and an appraisal of the phyletic status of the Trichomycterinae (Teleostei, Trichomycteridae). Amer. Mus. Novit., 2950: 1-39.

— 1992. A new subfamily of Trichomycteridae (Teleostei, Siluriformes), lower loricarioid relationships and a discussion on the impact of additional taxa for phylogenetic analysis. J. Linn. Soc., Zool., 106: 175-229.

— 1998. Phylogenetic relationships of Neotropical Siluriformes (Teleostei: Ostariophysi); historical overview and synthesis of hypothesis. Pp. 279-330 in: L. Malabarba, R. Reis, R. P. Vari, C. Lucena & M. Lucena (eds.), Phylogeny and classification of neotropical fishes. Museu de Ciências e Tecnologia – PUCRS, Porto Alegre, Brazil.

Proudlove, G. S. 2001. The conservation status of hypogean fishes. Env. Biol. Fishes, 62: 201-213.

Sket, B. 1988. Speleobiological investigations in the Colombian Andes. Biol. Vestnik, 36: 53-62.

Taylor, W. R. & G. A. Van Dyke. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium, 9: 107-119.

Tchernavin, V. 1944. A revision of some Trichomycterinae based on material preserved in the British Museum (Natural History). Proc. Zool. Soc. London, 114: 234-275.

Trajano, E. 1995. Evolution of tropical troglobites: applicability of the model of Quaternary climatic fluctuations. Mém. Biospéol., 22: 203-209.

— 1997. Synopsis of Brazilian troglomorphic fishes. Mém. Biospéol., 24: 119-126.

— 2000. Cave faunas in the Atlantic tropical rain forest: composition, ecology and conservation. Biotropica, 32: 882-893.

— 2001. Ecology of subterranean fishes: an overview. Env. Biol. Fish., 62: 133-160.

Trajano, E. & M. E. Bichuette. 2003. Área cárstica de São Domingos, Alto Tocantins, nordeste de Goiás: a maior diversidade de peixes subterrâneos no Brasil. O Carste, 15: 114-125.

Trajano, E. & F. A. Bockmann. 1999. Evolution of ecology and behaviour in Brazilian cave Heptapterinae catfishes, based on cladistic analysis (Teleostei: Siluriformes). Mém. Biospéol. 26: 123-129.

Trajano, E. & M. C. C. de Pinna. 1996. A new cave species of *Trichomycterus* from eastern Brazil (Siluriformes, Trichomycteridae). Rev. Fr. Aquariol., 23: 85-90.

Trajano, E., R. Reis & M. E. Bichuette. 2004. *Pimelodella spelaea*: a new cave catfish from Central Brazil, with data on ecology and evolutionary considerations. Copeia, 2004: 315-325.

Weber, A., G. S. Proudlove, J. Parzefall, H. Wilkens & T. T. Nalbant. 1998. Pisces (Teleostei). Pp. 1179-1190 in: C. Juberthie & V. Decu (eds.), Encyclopaedia biospeologica, Tome II. Société de Biospéologie, Moulis, France.

Wilkens, H. 1982. Regressive evolution and phylogenetic age: the history of colonization of freshwaters of Yucatan by fish and crustacea. Texas Mem. Mus. Bull., 128: 237-243.

— 1986. The tempo of regressive evolution: studies of the eye reduction in stygobiont fishes and decapod crustaceans of the Gulf Coast and West Atlantic region. Stygologia, 2: 131-143.

Wosiacki, W. 2002. Estudo das relações filogenéticas de Trichomycterinae (Teleostei, Siluriformes, Trichomycteridae) com uma proposta de classificação. Ph. D. Thesis, Universidade de São Paulo, 324 pp.

Received 6 March 2003
Revised 19 March 2004
Accepted 18 May 2004

Figure 1a

Figure 4a

Figure 7a