The Laterosensory Canal System in Epigean and Subterranean *Ituglanis* (Siluriformes: Trichomycteridae), With Comments About Troglomorphism and the Phylogeny of the Genus

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ABSTRACT The laterosensory system is a mechanosensory modality involved in many aspects of fish biology and behavior. Laterosensory perception may be crucial for individual survival, especially in habitats where other sensory modalities are generally useless, such as the permanently aphotic subterranean environment. In the present study, we describe the laterosensory canal system of epigean and subterranean species of the genus *Ituglanis* (Siluriformes: Trichomycteridae). With seven independent colonizations of the subterranean environment in a limited geographical range coupled with a high diversity of epigean forms, the genus is an excellent model for the study of morphological specialization to hypogean life. The comparison between epigean and subterranean species reveals a trend toward reduction of the laterosensory canal system in the subterranean species, coupled with higher intraspecific variability and asymmetry. This trend is mirrored in other subterranean fishes and in species living in different confined spaces, like the interstitial environment. Therefore, we propose that the reduction of the laterosensory canal system should be regarded as a troglomorphic (= cave-related) character for subterranean fishes. We also comment about the patterns of the laterosensory canal system in trichomycterids and use the diversity of this system among species of *Ituglanis* to infer phylogenetic relationships within the genus. J. Morphol. 000:000–000, 2016. © 2016 Wiley Periodicals, Inc.

KEY WORDS: lateral line; troglobite; cavefish; cave adaptation; morphological evolution

INTRODUCTION

The neuromast-based mechanoreceptive laterosensory system in teleosts (*sensu* Webb, 1989) consists of lines of sensory organs, the neuromasts, which detect and react to mechanical stimuli produced by water displacement (Dijkgraaf, 1962; Montgomery et al., 1995; McHenry and Liao, 2014). Such structures may be present in shallow pits or grooves in the skin surface (the superficial neuromasts), or inside pored, subepidermal canals. The canals are ususally embedded in dermal bones or are surrounded by bony tubes, opening to the surface through pores (the laterosensory canal system; Webb, 2014). The laterosensory system is involved in a series of behaviors (Bleckmann, 1986; Montgomery et al., 2014) and may represent a crucial resource for detection of environmental stimuli in the absence of other sources of sensory information, as is seen in fishes living in subterranean habitats or other aphotic environments.

Trichomycteridae (Teleostei: Ostariophysi: Siluriformes; Fig. 1) is the third richest family in terms of number of known exclusively subterranean fishes (Rizzato and Bichuette, 2014). The family is a monophyletic assemblage of small catfishes easily diagnosed by their highly specialized opercular-interopercular apparatus bearing odontodes (de Pinna, 1998; de Pinna and Wosiacki 2003; Adriaens et al., 2010; Datovo and Bockmann, 2010). Species in this clade show a high diversity of diet types and life habits (Datovo and Bockmann, 2010), including parasitic or semi-parasitic habits (especially in the more derived subfamilies, for example, the mucus and scale-eating stegophilines and the hematophagous vandellines), and the

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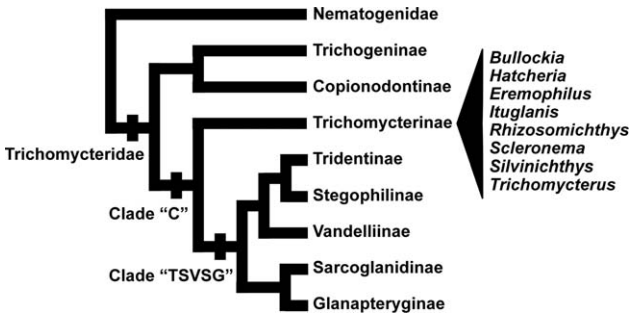

Fig. 1. Cladogram depicting the relationships between the subfamilies of Trichomycteridae, as well as the composition of the clades "C" and "TSVSG" that are refered to in literature and in the present work. The topology was based on the work from Datovo and Bockmann, Neotrop Ichthyol, 2010, 8, 193–246, reproduced by permission. The eight genera of Trichomycterinae are highlighted on the inset.

successful colonization of unusual habitats, such as the subterranean environment (Rizzato and Bichuette, 2014).

All but two of the known subterranean species of trichomycterids are included in its most speciose subfamily, the Trichomycterinae (*sensu* Datovo and Bockmann, 2010; Fig. 1), which includes the genera *Bullockia, Eremophilus, Hatcheria, Rhizosomichthys, Scleronema, Silvinichthys*, and the most species-rich *Trichomycterus* and *Ituglanis*. The genus *Trichomycterus* is also the richest trichomycterid genus in the number of known subterranean representatives, with 13 species distributed in caves from Brazil, Colombia, Venezuela, and Bolivia, followed by *Ituglanis*, with seven subterranean species known to date, all of which occur in Brazilian caves (Rizzato and Bichuette, 2014).

The seven subterranean species of *Ituglanis*: *I. passensis*, *I. bambui*, *I. epikarsticus*, *I. ramiroi*, *I. mambai*, *I. boticario*, and an undescribed species from caves in the Posse municipality (Goiás state, Brazil), *Ituglanis* sp. "Posse", are of special interest for both geographical and evolutionary reasons. These species occur in a very limited geographic range, the northeastern portion of Goiás state, Brazil, where two large carbonate (= limestone) karst areas occur, the São Domingos, to the north, and the Mambaí, to the south (see map and details in Rizzato and Bichuette, 2014). There is evidence that the colonization of the subterranean environment occurred independently for each of these species. Collectively, they show a mosaic of troglomorphic (= cave-related) features with the overall more troglomorphic species, *I. passensis, I. bambui, I. epikarsticus*, and *I. ramiroi* occuring in the São Domingos karst area, while the less troglomorphic species, *I. mambai*, *I. boticario*, and *Ituglanis* sp. "Posse" occur in the Mambaí karst area. This special situation places these species in the focus of studies of convergent evolution of cave-related characters in subterranean fishes.

Arratia and Huaquin (1995) described the laterosensory canals of representatives of most genera of Trichomycterinae, except for *Ituglanis*. Since the work of Datovo and Landim (2005), descriptions of the laterosensory canals and pores have been included in taxonomic works for all new species of *Ituglanis* described or redescribed so far: *I. macunaima* (Datovo and Landim, 2005); *I. cahyensis* (Sarmento-Soares et al., 2006); *I. paraguassuensis* (Campos-Paiva and Costa, 2007); *I. amazonicus*, *I. parkoi*, plus four undescribed species from the Amazon basin (Canto, 2009); *I. ina* (Wosiacki et al., 2012); *I. agreste* (Lima et al., 2013); *I. apteryx* (Datovo, 2014); *I. australis* (Datovo and de Pinna, 2014); *I. boticario* (Rizzato and Bichuette, 2014); *I. boitata* (Ferrer et al., 2015); and *I. goya* (Datovo et al., 2016). Sarmento-Soares et al. (2006) also provide a description of the laterosensory canal system of *I. proops* and *I. parahybae*. Some of these studies use the pattern of canals and pores of the laterosensory system to diagnose species (Datovo and Landim, 2005; Sarmento-Soares et al., 2006; Lima et al., 2013; Datovo, 2014; Datovo and de Pinna, 2014; Rizzato and Bichuette, 2014; Ferrer et al., 2015; Datovo et al., 2016).

In the present work, we describe the general pattern of laterosensory canals for *Ituglanis*, comment about the diversity of this system among epigean and subterranean species, and analyze the intraspecific and intraindividual variability with respect to canals and segments of canals, especially in subterranean species. With these data, and using *Ituglanis* as a model, we discuss the relationship between the variability and reduction of the laterosensory canal system and the subterranean life habit of fishes in general. We also include comments about the phylogenetic relationships among the species of *Ituglanis*, using inferences provided by the diversity of the laterosensory csnal system within the genus.

MATERIALS AND METHODS

We collected data about the laterosensory system of canals for 24 of the 26 described species of *Ituglanis*—the exceptions were *I. guayaberensis* (Dahl, 1960) and *I. laticeps* (Kner, 1863)—including the six described subterranean species, plus 1 undescribed subterranean species (*Ituglanis* sp. "Posse"). Additionally, we included data from three species of *Trichomycterus*: *T. brachykechenos* (Ferrer and Malabarba, 2013); *T. cachiraensis* (Ardila-Rodríguez, 2008); and *T. steindachneri* (DoNascimiento et al., 2014a,b). These three species were recently proposed to be closely related to the genus *Ituglanis* (DoNascimiento et al., 2014b), based on a supposed morphocline in relation to the reduction of the cranial fontanel, and they are included here as an outgroup for comparison.

Data from species of *Ituglanis* were obtained from direct examination of alcohol-preserved and/or stained (= enzymatically cleared and double-stained for bone and cartilage according to the procedures described by Taylor and Van Dyke, 1985; Song and Parenti, 1995, until step 9; and Datovo and Bockmann, 2010) specimens (Table 1). Data from the remaining

TABLE 1. Comparative material

Species	Register no.	*N*	SL (mm)	Examination method	
				Alc.	St.
Order Polypteriformes					
Family Polypteridae					
Polypterus senegalus (*N* = 4)	LIRP10236	1	184	1	
	LIRP4573	1	135.7	1	
	LIRP7426	2	64.8–65.2		2
Order Lepisosteiformes					
Family Lepisosteidae					
Lepisosteus osseus (*N* = 3)	LIRP9137	1	96.2	1	
	LIRP9138	1	75.0		1
	LIRP9140	1	136.8	1	
Order Amiiformes					
Family Amiidae					
Amia calva (*N* = 11)	LIRP4576	2	200–210	2	
	LIRP9139	3	51.0–60.9	2	1
	MZUSP194454	1	70.0		1
	MZUSP46123	5	30.0–40.0		5
Order Siluriformes					
Family Diplomystidae					
Dyplomistes mesembrinus (*N* = 2)	LIRP11282	2	82.0–102.9	1	1
Family Nematogenyidae					
Nematogenys inermis (*N* = 2)	LIRP11283	2	62.1–76.4	1	1
Family Trichomycteridae					
Subfamily Trichogeninae					
Trichogenes longipinnis (*N* = 6)	LIRP1039	6	29.9–73.5	3	3
Subfamily Copionodontinae					
Copionodon pecten (*N* = 26)	LIRP1012	26	19.5–57.0	22	4
Subfamily Trichomycterinae					
Trichomycterus immaculatus (*N* = 16)	LIRP285	16	32–92.3	13	3
T. paolence (*N* = 22)	LIRP8281	22 of 145	24–85.3	22	
T. brasiliensis (*N* = 22)	LIRP9742	22 of 67	33.4–77.3	22	
I. amazonicus (*N* = 16)	MCP36257	8 of 13	23.8–52.7	8	
	MZUSP86804	2	30.4–35.4	2	
	MZUSP86821	6 of 8	24.2–45	6	
I. australis (*N* = 19)	UFRGS13600*	1	75.1	1	
	UFRGS12578*	1	39.8	1	
	MZUSP112505**	1	57.1	1	
	MZUSP081016	1	36.7	1	
	MZUSP112507	2	43.0–44.1	2	
	MZUSP112506	1 of 2	28.2	1	
	UFRGS7444*	1	63.4	1	
	MCP10420*	4	39.9–51.0	3	1
	UFRGS11237*	1	64.0	1	
	MCP37695*	6 of 7	52.0–59.0	4	2
I. bambui (*N* = 46)	MZUSP79860**	1	42.1	1	
	MZUSP79861	1	34.0	1	
	MZUSP79862*	3 of 4	30.6–45.5	3	1
	MZUSP79863*	3	35–41.5	2	1
	MZUSP79864*	4	31.7–46.3	2	2
	LESCI00004	2	16.6–22.4	2	
	LESCI00009	2	34.3–34.4	2	
	LESCI00010	7	21.3–34.6	7	
	LESCI00034	6	29.2–39.8	5	1
	LESCI00117	6	29.1–48.9	5	1
	LESCI00151	10	31.6–49.4	8	2
I. boticario (*N* = 14)	LIRP11009**	1	69.7	1	
	LIRP11010*	4	47.1–68.9	3	1
	LIRP11011*	1	76.5		1
	LIRP11012*	3	29.1–62.8	3	
	LIRP11013*	1	46.1	1	
	LESCI00041*	1	53.9	1	
	LESCI00223*	3	55.3–73.5	2	1
I. epikarsticus (*N* = 5)	MZUSP79869**	1	25.9	1	

Table 1. (continued).

Species	Register no.	*N*	SL (mm)	Examination method	
				Alc.	St.
	MZUSP79872*	1	29.9		1
	MZUSP79871*	2	29.2–30.6		2
	LESCI00300	1	45.7		1
I. goya (*N* = 95)	MCP15911	5 of 25	30.2–51.1	5	
	MCP15930	32 of 37	33.2–59.1	30	2
	MCP15938	5 of 12	37.6–51.4	4	1
	MZUSP40716	2	41.7–47.8	2	
	MZUSP40790	1	46.6	1	
	MZUSP40792	11	41.9–54.8	5	
	MZUSP40798	1	54.2	1	
	MZUSP40805	1	48.4	1	
	MZUSP40814	2	50.2–56.9	2	
	MZUSP53976	1	76.6		1
	UFRGS11193	1	46	1	
	UFRGS11198	1	66.3	1	
	UFRGS11202	12 of 17	29.4–74.5	11	1
	UFRGS11215	1	37.8	1	
	UFRGS11222	2	39.4–46.4	1	1
	UFRGS11237	6	30.8–43.7	5	1
	UFRGS11297	7	33.3–59.1	7	
	UFRGS11336	3	37.4–39	3	
	UFRGS11365	1	46.4	1	
I. herberti (*N* = 13)	NUP2242	2	56.9–88.1	2	
	MNRJ1429****	3 of 5	50.0–69.0	3	
	NUP2240	2	48.0–58.0	2	
	NUP2241	2 of 3	63.0–64.0	2	
	NUP2238	1	34.9	1	
	MZUSP59387	2	37.0–74.5	2	
	NUP3187	1	48.0	1	
I. ina (*N* = 9)	MZUSP107065	1	26.8	1	
	MZUSP107066	1	23.39	1	
	MZUSP uncat.	7	45–58.1	7	
I. macunaima (*N* = 26)	MZUSP86272*	1	26.8	1	
	MZUSP88452**	1	30.2	1	
	MZUSP86237*	5	24.0–31.2	4	1
	MZUSP86251	1 of 2	22.7	1	1
	MNRJ780***	1 of 2	24.5	1	
	MCP36244	1	33.8	1	
	MCP36243	1	26.7	1	
	MZUSP90202	1	31.5	1	
	MZUSP37640	3	40.0–44.5	3	
	MZUSP95014	10 of 11	28.9–38.0	10	
I. mambai (*N* = 14)	MZUSP94719*	4	26.6–66.1	4	
	MCP42537*	1 of 3	57.9	1	
	LISDEBE2047*	6	32.7–64.7	6	
	LESCI00239	3	53.1–66.4	3	
I. metae (2)	MZUSP105804	2	22.8–53.7	2	
I. paraguassuensis (*N* = 21)	MZUSP88550	1	41.8	1	
	MZUSP88553	7	25.8–32.2	7	
	MZUSP63138	1	53.9	1	
	MZUSP102535	6	37.5–44.8	6	
	MZUSP88555	2	36.5–39.6	2	
	MZUSP102703	3 of 5	41.0–43.0	3	
	MZUSP88554	1	31.6	1	
I. parahybae (*N* = 10)	MZUSP115069	3	52.0–64.1	3	
	MZUSP71852	3	36.0–41.2	3	
	MCP18026	1	36.3	1	
	MCP18032	1	47.8	1	
	MCP17784	1	46.2	1	
	MZUSP79810	1 of 2	41.0	1	
I. passensis (*N* = 34)	MZUSP80099	2	50.7–53.9	2	
	MCP27436	3	29,5–65.2	3	

Table 1. (continued).

Species	Register no.	*N*	SL (mm)	Examination method	
				Alc.	St.
	MZUSP80098	2 of 3	27.1–49.1	2	
	MZUSP80097	2 of 3	56.2–57.1	2	
	MCP27436	3	30.5–65.8	3	
	LESCI00008	19	30.4–60.1	17	2
	LESCI00016	1	15.6	1	
	LESCI00019	1	15.4	1	
	LESCI00189	1	30.5	1	
I. proops (*N* = 38)	MNRJ781***	1 of 3	64.0	1	
	MZUSP79576	15 of 30	40.4–65.8	15	
	LIRP9313	18 of 20	45.6–82.2	17	1
	MZUSP83752	4	47.9–49.5	2	
	MZUSP70725	11 of 15	92.6		
	MZUSP60255	2	57,1–77.0		2
I. ramiroi (*N* = 25)	MZUSP79865**	1	27.1	1	
	MZUSP79867	3	29.2	1	2
	MZUSP79868	1	29.2	1	
	LESCI00006	2	34.9–36.6	1	1
	LESCI00150	5	32.1–47.8	4	1
	LESCI00188	1	41.3		1
	LESCI00214	1	35	1	
	LESCI00215	6 of 8	28.1–38.7	6	
	LESCI 00328	5	37.1–49.1	5	
Ituglanis sp. "Posse" (*N* = 33)	LESCI00046	1	49.8	1	
	LESCI00047	1	35.1	1	
	LESCI00216	1	47.4	1	
	LESCI00217	3	47–59	3	
	LESCI00218	2	53.6	1	1
	LESCI00219	1	45.7	1	
	LESCI00220	2	46.7–48.7	1	1
	LESCI00221	1	65.8	1	
	LESCI00222	1	37.5	1	
	LESCI00237	2	37.2–32.4	2	
	LESCI00240	3	45.7–62.9	3	
	LESCI00257	15	28.5–51.3	14	1

N = Number of specimens analyzed. SL = Standard length. Alc. = Specimen in alcohol. St. Stained specimen. * Paratype. ** Holotype. *** Lectotype.

species were obtained from the literature (Table 2). The number, distribution, and position of external openings of the laterosensory canals (i.e., pores) were analyzed for the alcohol-preserved specimens, while the stained specimens were used to analyze the presence and course of the canals. The presence/absence of pores in alcohol-preserved specimens was used to infer the presence/absence of the respective laterosensory canals and, in some specimens, this was later confirmed after staining. Figure 4 is based on stained and dissected specimens and Figures 5–7 are based on cleared and stained specimens.

Material Analyzed

See Table 1. Institutional abbreviations. LESCI = Coleção de Ictiologia, Laboratório de Estudos Subterrâneos, Departamento de Ecologia e Biologia Evolutiva (DEBE), Universidade Federal de São Carlos, São Carlos, SP, Brazil. LIRP = Laboratório de Ictiologia de Ribeirão Preto, Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Ribeirão Preto, SP, Brazil. LISDEBE = Laboratório de Ictiologia Sistemática, DEBE, Universidade Federal de São Carlos, São Carlos, SP, Brazil. MCP = Pontifícia Universidade Católica do Rio Grande do Sul, Museu de Ciências e Tecnologia, Porto Alegre, RS, Brazil. MNRJ = Museu Nacional, Departamento de Vertebrados, Setor de Ictiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil. MZUSP = Universidade de São Paulo, Museu de Zoologia, São Paulo, SP, Brazil. NUP = Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura, Universidade Estadual de Maringá, Maringá, PR, Brazil. UFRGS = Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Nomenclature

The nomenclature of the skeleton in *Amia calva* follows Grande and Bemis (1998) and for catfishes follows Datovo and Bockmann (2010). The identification of the laterosensory canals (Fig. 2) was based on a comparison with the condition in *Amia calva* (Holostei: Amiiformes), a basal actinopterygian that represents the plesiomorphic, generalized condition of the laterosensory system in the crown-group Actinopterygii, described in details by Allis (1889), and in *Nematogenys inermis* (Siluriformes: Nematogenyidae), considered by many authors the sister-group of Trichomycteridae (e.g., Datovo and Bockmann 2010 and references therein). Consequently, the nomenclature of canals used here for *Ituglanis* disagrees in part with previous usages, which for the most part are based on work by Arratia and Huaquin (1995). The differences are described in the Supporting Information.

According to Allis' (1889, p. 467, cut. 1) description of the developmental stages for the formation of the laterosensory

TABLE 2. Pattern of laterosensory canals in species of *Ituglanis* and in the three species of *Trichomycterus* suggested as closely related to the genus (*DoNascimiento et al., 2014a,b*)

	Rib count	ASI	N	N+F	F	S	O	Po	Sc	T
T. steindachneri	14–18	P	P	P	P	P	P	P	P	P
T. cachiraensis	12	P	P	P	P	P	P	P	P	P
T. brachykechenos	12		P	P	P	P	P	P	P	P
I. agreste	5–6	P	P	P	P	P	P	P	P	P
I. paraguassuensis	6	P	P	V	P	P	P	P	P	P
I. boitata	5–6	P	P		P	P	P	P	P	P
I. proops	4–7	P	P		P	P	P	P	P	P
I. australis	4–6	V		n/a	P	P	P	P	P	P
I. boticario	7–8	P	P	V	P	P	P	P	P	P
I. mambai	6–10	V	P	V	P	P	P	P	P	P
Ituglanis sp. "Posse"	5–9	V	P	V	P	P	P	P	P	P
I. bambui	5–9		V		V	P	P	P	P	P
I. ramiroi	5–8		V		V	V	P	P	P	V
I. passensis	5–8			n/a			P	P	P	V
I. epikarsticus	5			n/a			V	P	P	
I. goya "Posse"	4–8		P	P	P	P	P	P	P	P
I. cahyensis	4			n/a	P	P	P	P	P	P
I. parahybae	6			n/a		P	P	P	P	P
I. laticeps	4	?	?	?	?	?	?	?	?	?
I. amazonicus	2–3		P	P	P	P	P	P	P	P
I. apteryx	2		P	P	P	P	P	P	P	P
I. gracilior	2–3		P	P	P	P	P	P	P	P
I. herberti	1–3		P	P	P	P	P	P	P	P
I. metae	1–3		P	P	P	P	P	P	P	P
I. parkoi	3		P	P	P	P	P	P	P	P
I. eichorniarum	2–3		P	V	P	P	P	P	P	P
I. ina	2–3		P	V	P	P	P	P	P	P
I. nebulosus	3		P	?	P	P	P	P	P	P
I. macunaima	2–3			n/a	P	P	P	P	P	P
I. guayaberensis	?	?	?	?	?	?	?	?	?	?

Species of *Ituglanis* divided into two groups, those with four or more pairs of ribs, and those with three or less pairs of ribs, and listed within the groups in an order that facilitates the visualization of the table and reflects ideas about phylogenetic proximity (see "comments on *Ituglanis* phylogeny"). Subterranean species highlighted in gray. The presence or absence of canals are indicated by "P" (= presence) and by an empty cell, respectively, while a question mark (?) indicates lack of information. Canals marked with "V" are variably present. When the nasal or the frontal canal is absent, it is impossible to know if in the ancestor condition these canals were fused or interrupted; these cases are indicated as "n/a" (non-applicable). ASI = antorbital segment of infraorbital canal. F = frontal canal. N + F = nasal and frontal canals continuous to each other. N = nasal canal. O = otic canal. Po = postotic canal. S = sphenotic canal. Sc = scapular canal. T = trunk canal.

canals in *Amia*, we decided to adopt the term "half-pore" to the two openings left on either side of a canal neuromast when it is enclosed by a canal segment. We also restricted the term "pore" to refer only to the definitive opening formed when the development of the canals is completed. As Allis (1889) demonstrated, the definitive opening to the surface of the skin seen in the adult may represent either a half-pore, or a pore formed by the fusion of two half-pores (more commonly), or even a fusion of three (a pore of triple origin) or more half-pores.

We refrained from using the names of individual pores as used by Arratia and Huaquin (1995), as it can cause confusion about the identity of the pores. For example, when four pores are present in the supraorbital line of canals, the second rostralmost is usually named "s2" and the third "s3." However, in many species of trichomycterids, including some *Ituglanis*, as described below, the second rostralmost pore of the supraorbital line of canals (the posterior half-pore of the nasal canal) may be fused to the third rostralmost pore (the rostralmost half-pore of the frontal canal) forming a single pore. In these cases, the correct naming should be "s2" + "s3", but many authors name the single pore thus formed "s3," as if the "s2"-component was absent. To avoid such confusions, we use the names proposed by Arratia and Huaquin (1995) between quotation marks and only as references for comparison with the descriptions of these and other previous authors.

As first demonstrated by Pollard (1892), the trichomycterids share the apomorphic actinopterygian condition of having a single neuromast between two half-pores (Webb and Northcutt, 1997). Therefore, although we did not visualize the neuromasts themselves, we assumed that a single neuromast was present between each pair of half-pore, as described and illustrated for *Trichomycterus tenuis* by Pollard (1892; Fig. 5). We use the term "supranumerary pore" to refer to multiple pores that arise by bifurcation of the single tubule that leads from the canal to the skin surface at the site of the two half-pores belonging to adjacent canal segments. Therefore, there are no neuromasts between supranumerary pores.

RESULTS

Nasal and Frontal Canals

These canals are refered to in literature as a single canal named "supraorbital canal," but we interpret them to be two independent, distinct canals (see Supporting Information). When present, the nasal canal is restricted to a short tube with two half-pores, and was therefore interpreted

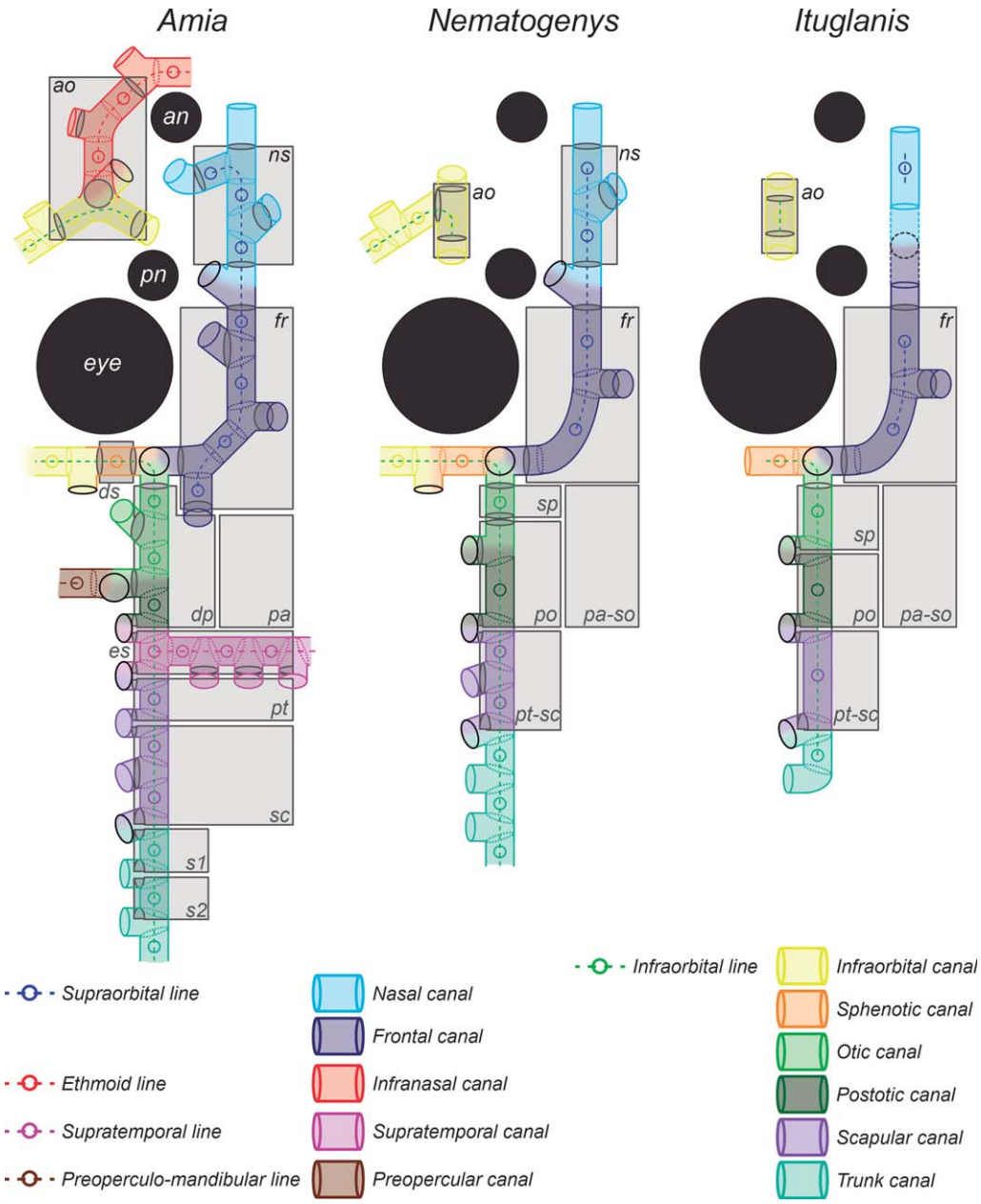

Fig. 2. Comparison between the laterosensory system of canals of *Amia calva*, *Nematogenys inermis* and a generalized *Ituglanis*, illustrating the nomenclature for canals used in this work. The diagrams represent the left side of the head of each species, in dorsal view. The diagram of *Amia* is based on Allis, J Morphol, 1889, 2, 462–566, reproduced by permission, but with new interpretations of the terminology of the canals (see Supporting information). The nomenclature of the skeleton in *Amia calva* follows Grande and Bemis, Society of Vertebrate Paleontology Memoir 4, Supplement to Journal of Vertebrate Paleontology, 1998, 18:iv+1-690, Society of Vertebrate Paleontology, reproduced by permission and for catfishes follows Datovo and Bockmann, Neotrop Ichthyol, 2010, 8, 193–246, reproduced by permission. As *Ituglanis* species lack the portion of the infraorbital canal that runs around the ventral margin of the orbit, that portion was omitted in the diagrams of *Amia* and *Nematogenys*, to easen the comparison. Pores marked in black are formed by half-pores of distinct, adjacent canals. an = anterior naris. ao = antorbital bone. dp = dermopterotic bone. ds = dermosphenotic bone. es = extrascapular bone. fr = frontal bone. ns = nasal bone. pa = parietal bone. pa-so = parieto-supraoccipital bone. pn = posterior naris. po = (auto)pterotic bone. pt = posttemporal bone. pt-sc = posttemporo-supracleithrum bone. sc = supracleithrum bone. sp = (auto)sphenotic bone.

as containing a single neuromast. The nasal canal lies just below the skin surface, anteroposteriorly directed, and medial to the nares (Figs. 3 and 4). The anterior half-pore ("s1") remains as a half-pore in the adult, and opens close to the medial margin of the anterior naris. The posterior one may remain as a half-pore (forming the "s2"), opening close to the medial margin of the posterior naris (e.g., *I. proops*, Figs. 3 and 4), or may fuse to the rostralmost pore of the frontal canal to form a

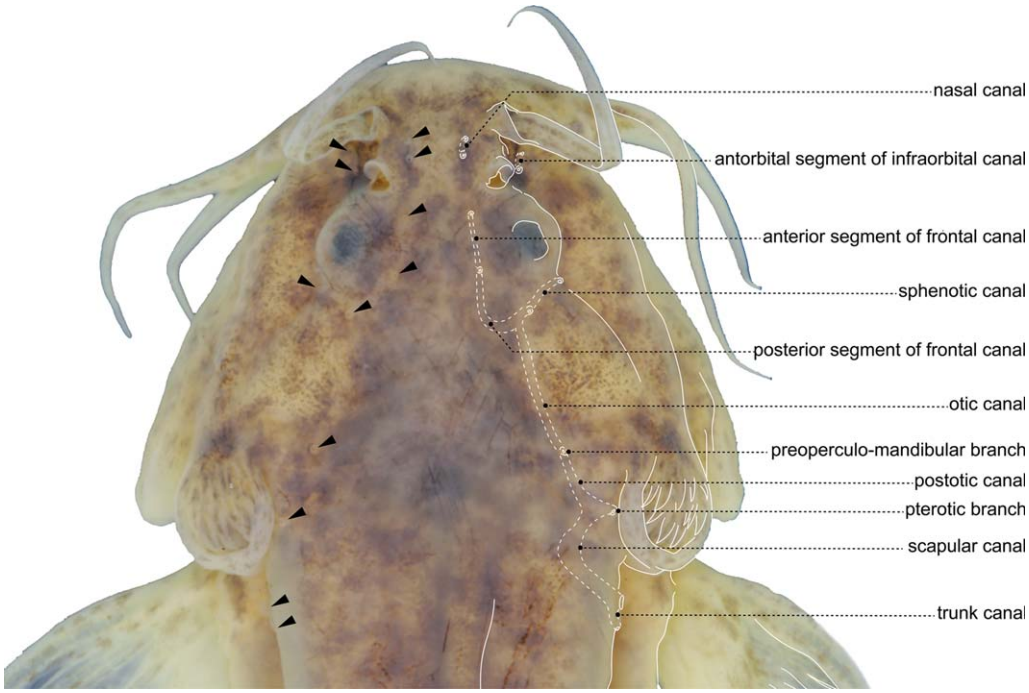

Fig. 3. *Ituglanis proops*, dorsal view of head (LIRP9313, 80.6 mm standard length), with laterosensory pores and subepidermal course of canals illustrated on the right. Arrowheads on the left point to the pores of the laterosensory canals.

single pore of double origin ("s2" + "s3"; e.g., *I. agreste* and *I. goya*, Fig. 5).

The frontal canal, when present, extends in an anteroposterior direction from the anterior margin of the frontal to the post-orbital process, which is formed by the dorsolateral process of frontal and the anterior process of sphenotic-prootic-pterosphenoid (Figs. 3 and 4). The frontal canal bears four half-pores, therefore contains two neuromasts. The first or rostralmost half-pore of the frontal canal ("s3") may remain as a half-pore, or may fuse to the posterior pore of the nasal canal to form a single pore of double origin ("s2" + "s3"). In both cases, it is located at the tip of a short tube exiting by an aperture at the anterior margin of the frontal. The second and third half-pores fuse together to form the middle pore of the frontal canal ("s6"), located approximately at the midlength of the canal, medially to the eyes. The fourth or caudalmost half-pore of the frontal canal is fused to the posterior or proximal half-pore of the sphenotic canal and the anterior pore of the otic canal, forming a single pore of triple origin ("i11").

When both nasal and frontal canals are present, they may be separated from each other, as in the epigean *I. proops* (Figs. 3 and 4) and *I. boitata*, or they may be fused to each other through the posterior half-pore of the nasal canal and the rostralmost half-pore of the frontal canal ("s2" = "s3") forming a continuous canal. In the epigean *I. eichorniarum, I. ina*, and *I. paraguassuensis* (Fig. 6) both conditions are variably present. Among the epigean species, the nasal canal is absent in *I. australis* (Fig. 5), *I. cahyensis*, and *I. macunaima* (Datovo and Landim, 2005; Sarmento-Soares et al., 2006; Datovo and de Pinna, 2014). In *I. parahybae*, both nasal and frontal canals are absent (Costa and Bockmann, 1993; Sarmento-Soares et al., 2006), therefore, the pore located behind the eye (the so-called "i11") is formed by the fusion of only the anterior half-pore of the otic canal and the posterior or proximal half-pore of the sphenotic canal.

Among the subterranean species of *Ituglanis*, the nasal and frontal canals, when present, are usually separated from each other, although in some specimens of *I. boticario, I. mambai*, and *Ituglanis* sp. "Posse", they may be fused (Fig. 6). In *I. bambui* and *I. ramiroi*, the nasal and frontal canals are variably present (Fig. 7). In many specimens of *I. ramiroi* and *I. bambui*, and in some specimens of *Ituglanis* sp. "Posse", the nasal canal is present and the frontal canal absent, or vice-versa. The frontal canal is reduced to its posterior segment in many specimens of *I. bambui* and *I. ramiroi*. The nasal and frontal canals are absent in the more troglomorphic *I. passensis* and *I. epikarsticus* (Fig. 7). A single specimen of *I. passensis* had the frontal canal present only on the left side of the head (Fig. 9).

Infraorbital Canal

When present, the infraorbital canal in *Ituglanis* is restricted to its rostralmost portion, which in

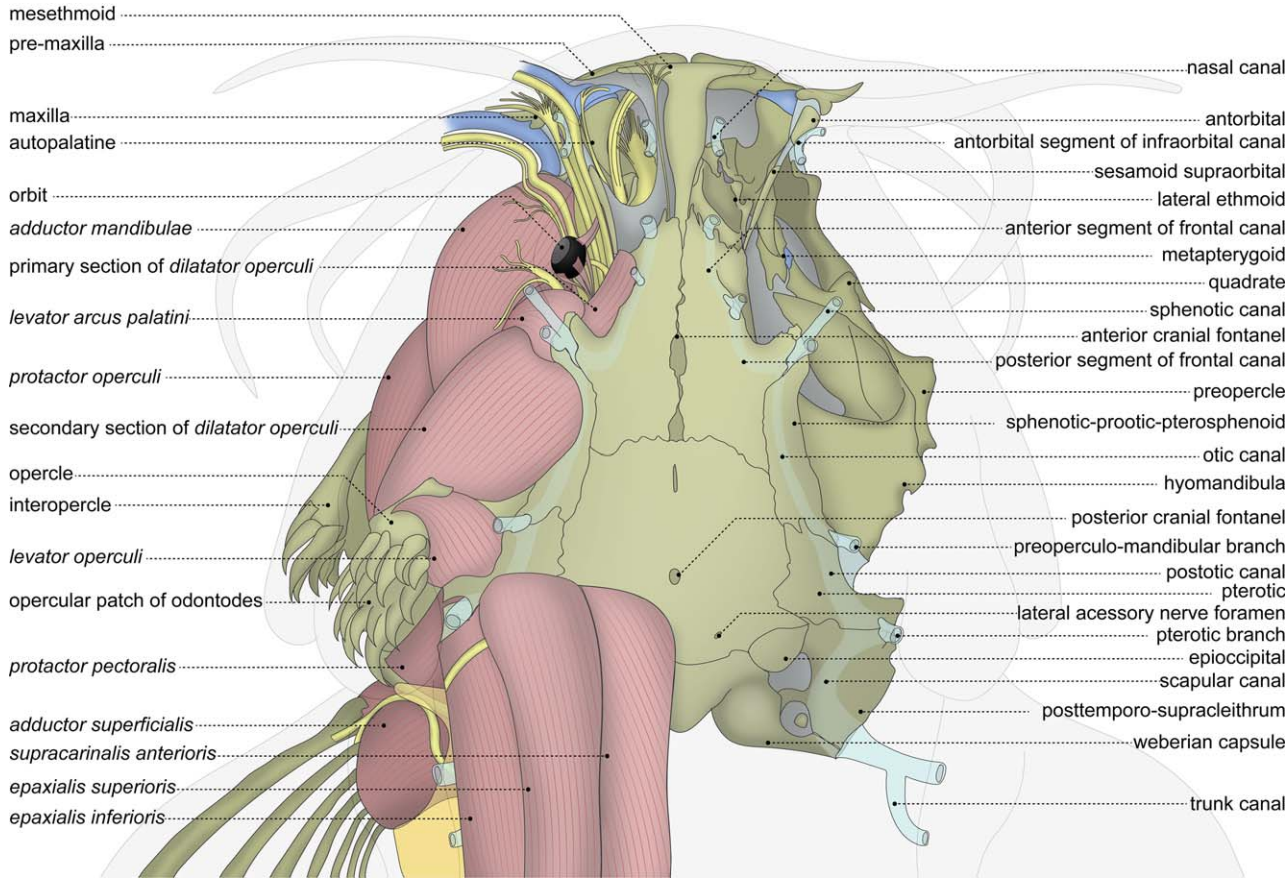

Fig. 4. *Ituglanis proops*, dorsal view of a partially dissected cephalic region, with muscles, main nerves, and other soft tissues illustrated on the left, showing the course of laterosensory canals through skin and bones. Opercle and interopercle not illustrated on the right side.

Amia, Nematogenys (Fig. 2), and many other fishes turns posteriorly and runs parallel to the axis formed by the anterior and posterior nares. This portion is identified as the antorbital portion of the infraorbital canal (see Supporting Information). In *Ituglanis*, the canal lies just below the skin surface, external to the nares, and is restricted to a short tube with two half-pores, therefore contains a single neuromast (Figs. 3 and 4). Both pores remain as half-pores in the adult, the anterior one ("i1") always closer to the anterior naris, and the posterior one to the posterior naris.

The infraorbital canal, represented only by its antorbital portion, is present only in five of the epigean species analyzed: *I. agreste* (Fig. 5), *I. boitata, I. proops* (Figs. 3 and 4), *I. paraguassuensis* (Fig. 6), and *I. australis* (Fig. 5), although in the latter it is variably present (Sarmento-Soares et al., 2006; Campos-Paiva and Costa, 2007; Lima et al., 2013; Datovo and de Pinna, 2014; Ferrer et al., 2015). In the remaining epigean species of the genus, the entire infraorbital canal is absent (Table 2).

Among the subterranean species, the antorbital portion of the infraorbital is present in about half of the specimens of *I. boticario*, but only in a few specimens of *I. mambai* and *Ituglanis* sp. "Posse", usually in only one side of the head (Fig. 6). In the more troglomorphic species, *I. bambui, I. ramiroi, I. passensis*, and *I. epikarsticus*, the antorbital portion is absent (Fig. 7). A single specimen of *I. bambui* had the antorbital portion present in both sides of the head (Fig. 9).

Sphenotic Canal

The sphenotic canal is short, with two half-pores, and was therefore interpreted as containing a single neuromast. The canal extends anterolaterally from the post-orbital process, which is formed by the dorsolateral process of frontal and the anterior process of sphenotic-prootic-pterosphenoid (Figs. 3 and 4). The distal half-pore of the canal ("i10") remains as a half-pore and lies external and caudal to the eye, while the proximal half-pore ("i11") lies caudal to the eye, close to the opening formed at the contact of the lateral process of frontal and the anterior process of sphenotic-prootic-pterosphenoid. The latter half-pore is fused to the anterior half-pore of the otic

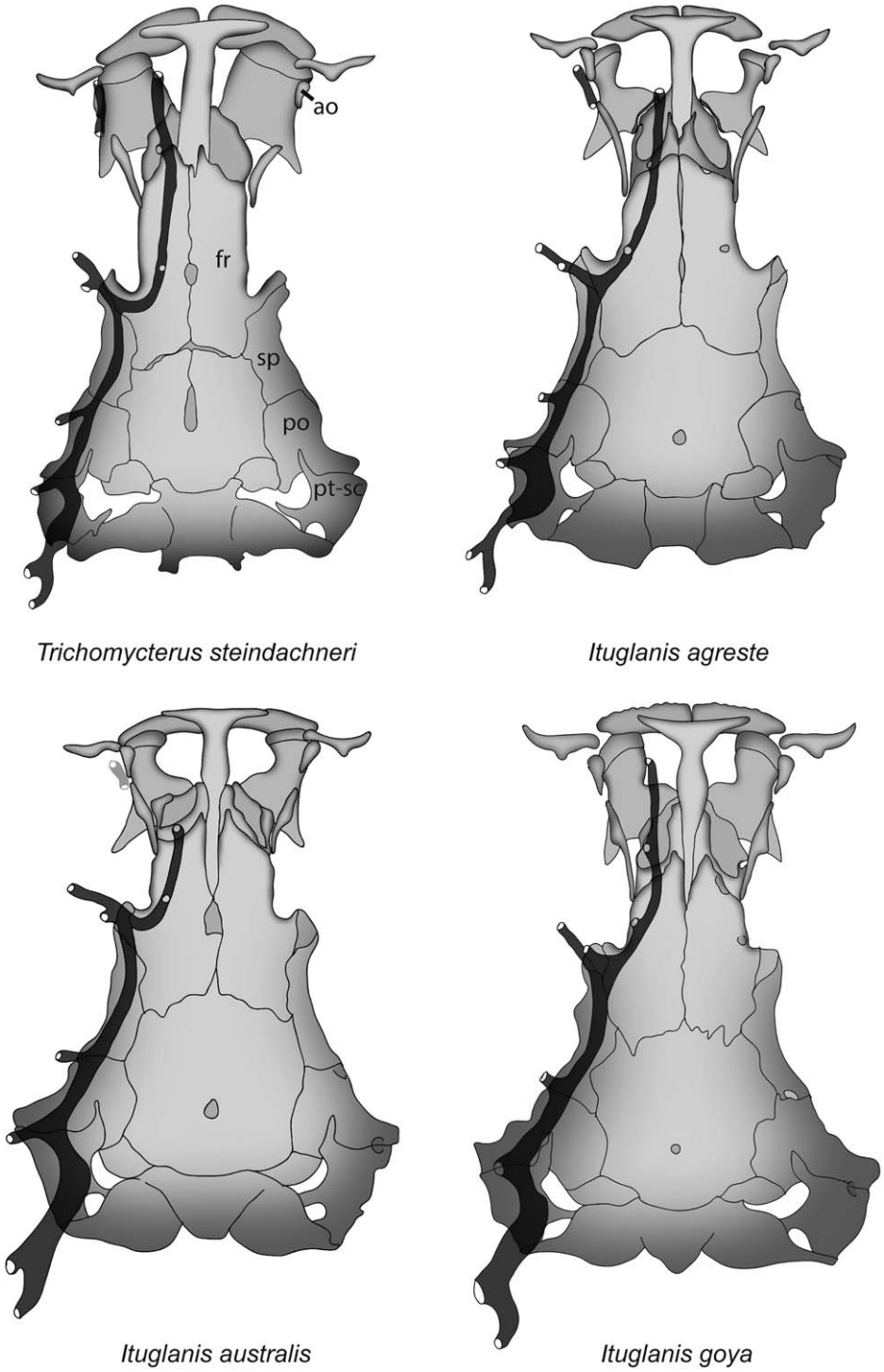

Fig. 5. Dorsal views of skulls of *Trichomycterus steindachneri* (modified from DoNascimiento et al., Neotrop Ichthyol, 2014b, 12, 717–728, reproduced by permission) and of three epigean species of *Ituglanis*, *I. agreste* (modified from Lima et al. Neotrop Ichthyol, 2013, 11, 513–524, reproduced by permission), *I. australis*, and *I. goya*. The course of the laterosensory canals and its pores are shown on the left side of each skull. ao = antorbital bone. fr = frontal bone. po = (auto)pterotic bone. pt-sc = posttemporo-supracleithrum bone. sp = (auto)sphenotic bone.

canal and the rostralmost half-pore of the frontal canal, forming a single pore of triple origin.

The sphenotic canal is present in all the epigean species of *Ituglanis* analyzed (Table 2). Among the subterranean species, it is variably present in *I. ramiroi* and absent in *I. passensis* and *I. epikarsticus* (Figs. 6 and 7). The sphenotic canal is absent in one specimen of *I. boticario* and in a few

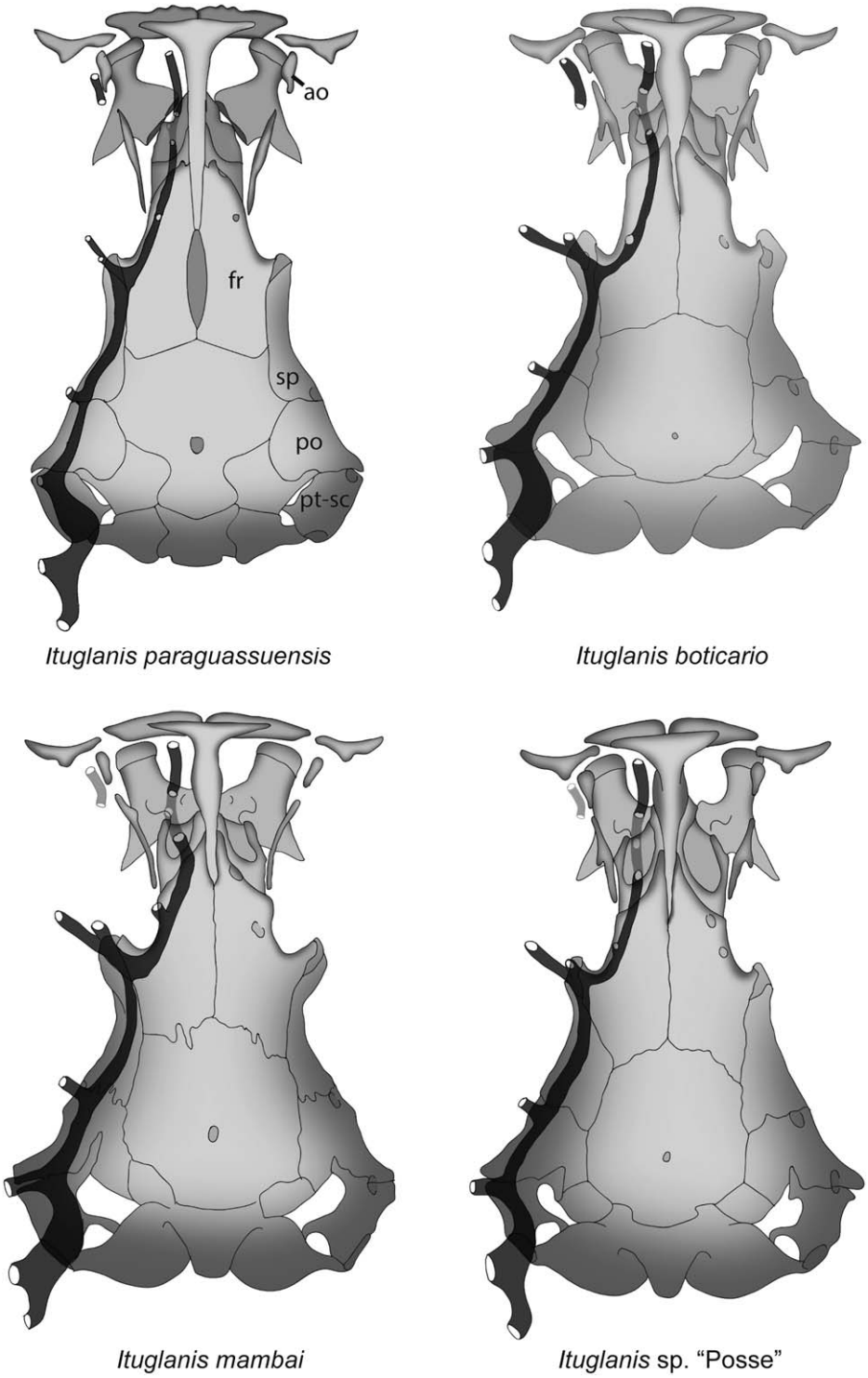

Fig. 6. Dorsal views of skulls of *Ituglanis paraguassuensis* (modified from Campos-Paiva and Costa, Zootaxa, 2007, 1471, 53–59, reproduced by permission) and of the three subterranean species of *Ituglanis* from Mambaí karst area, *I. boticario, I. mambai*, and *Ituglanis* sp. "Posse". The course of the laterosensory canals and its pores are shown on the left side of each skull. ao = antorbital bone. fr = frontal bone. po = (auto)pterotic bone. pt-sc = posttemporo-supracleithrum bone. sp = (auto)sphenotic bone.

specimens of *I. mambai* (Figs. 7–9). A single specimen of *I. passensis* had the sphenotic canal present in only one side of the head (Fig. 9).

Otic Canal

The otic canal in *Ituglanis* runs from the postorbital process to the posterodorsal contact

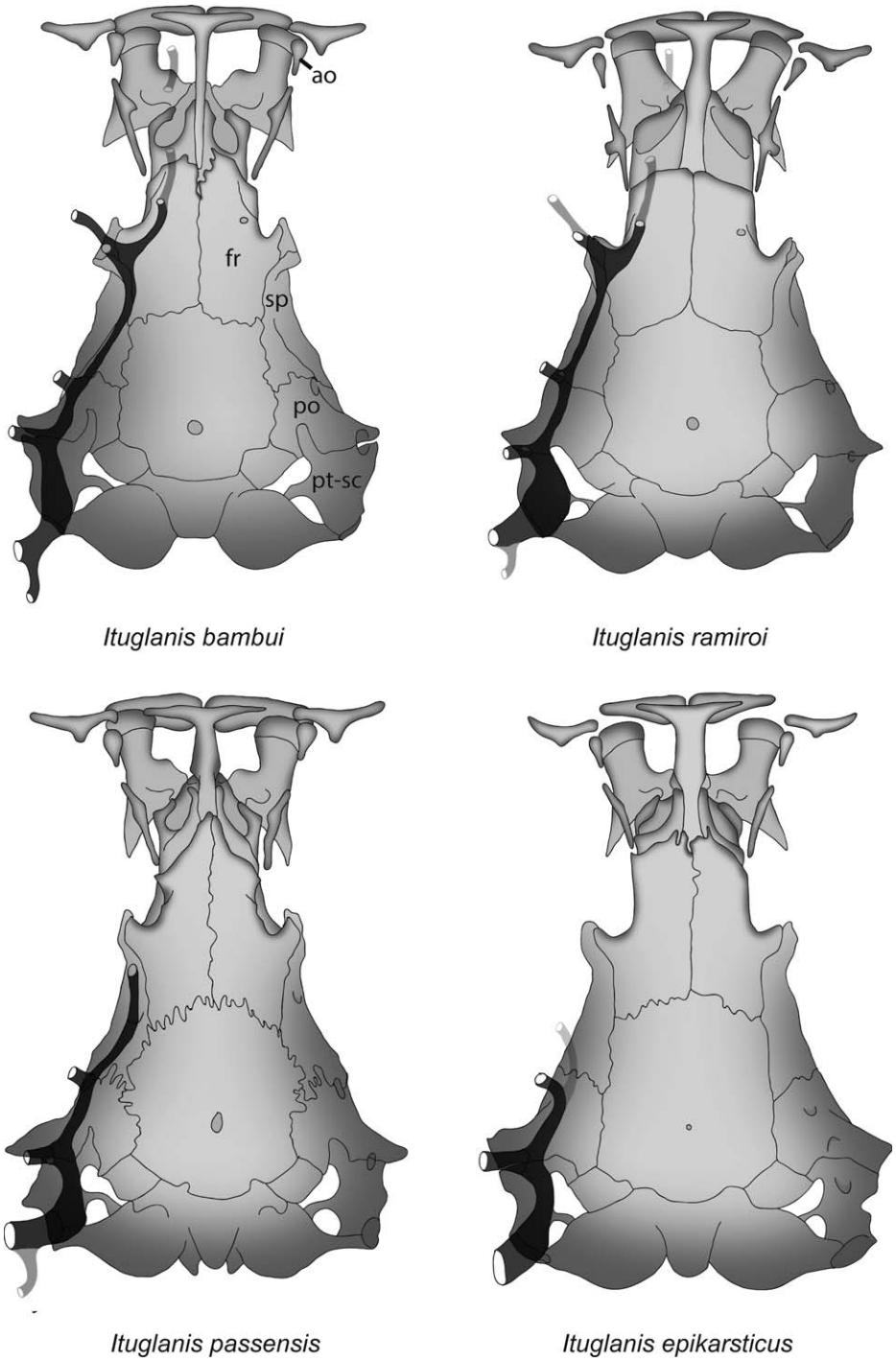

Fig. 7. Dorsal views of skulls of the subterranean species of *Ituglanis* from São Domingos karst area, *I. bambui*, *I. ramiroi*, *I. passensis*, and *I. epikarsticus*. The course of the laterosensory canals and its pores are shown on the left side of each skull. ao = antorbital bone. fr = frontal bone. po = (auto)pterotic bone. pt-sc = posttemporo-supracleithrum bone. sp = (auto)sphenotic bone.

between the sphenotic-prootic-pterosphenoid and the pterotic (Figs. 3 and 4). The otic canal is continuous posteriorly with the postotic canal, which means that the anterior half-pore of the postotic canal ("po1") is actually fused to the posterior half-pore of the otic canal. Similarly, the otic canal is continuous rostrally with the sphenotic and frontal canals, which means that the anterior half-pore of the otic canal is fused to the caudalmost half-pores of the sphenotic and frontal canals, forming a single pore of triple origin. Therefore, a single neuromast is present within the otic canal.

The otic laterosensory canal is present in all the epigean species of *Ituglanis* analyzed (Table 2).

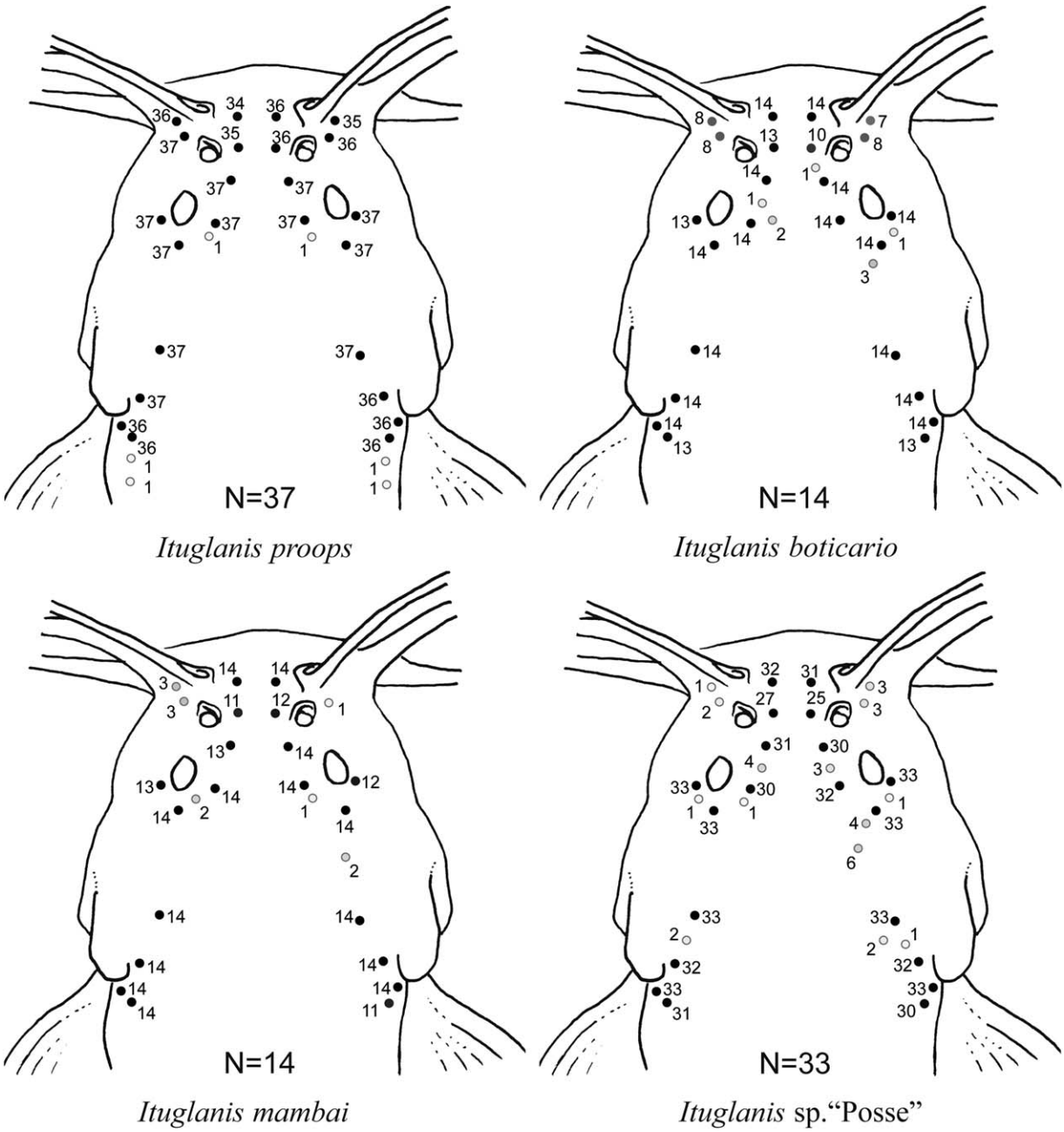

Fig. 8. Variability in the number of external openings of the laterosensory canals (i.e., pores) in the epigean *Ituglanis proops* and in the subterranean *I. boticario, I. mambai*, and *Ituglanis* sp. "Posse". The number adjacent to each pore (dots) refer to the number of specimens in which each pore is present among the total number of specimens analyzed (*N*). The darker the pore, the higher its frequency among the specimens.

Among the subterranean species, it is present in all specimens of *I. boticario, I. mambai, Ituglanis* sp. "Posse" and *I. ramiroi*, and in almost all specimens of *I. bambui* (Figs. 6 and 7). It is present in most specimens of *I. passensis* but shortened, with the pore opening more caudally on the head (Figs. 7 and 9). It is absent in *I. epikarsticus*, except in only one side of the head in two specimens (Figs. 7 and 9).

Postotic Canal

The postotic canal in *Ituglanis* bears two half-pores, therefore contains a single neuromast. It runs in an anteroposterior direction, enclosed in the dorsal portion of the pterotic (Figs. 3 and 4). The anterior half-pore opens at, or slightly caudal to, the dorsal contact between the pterotic and the sphenotic-prootic-pterosphenoid, being fused to the posterior pore of the otic canal; it represents the

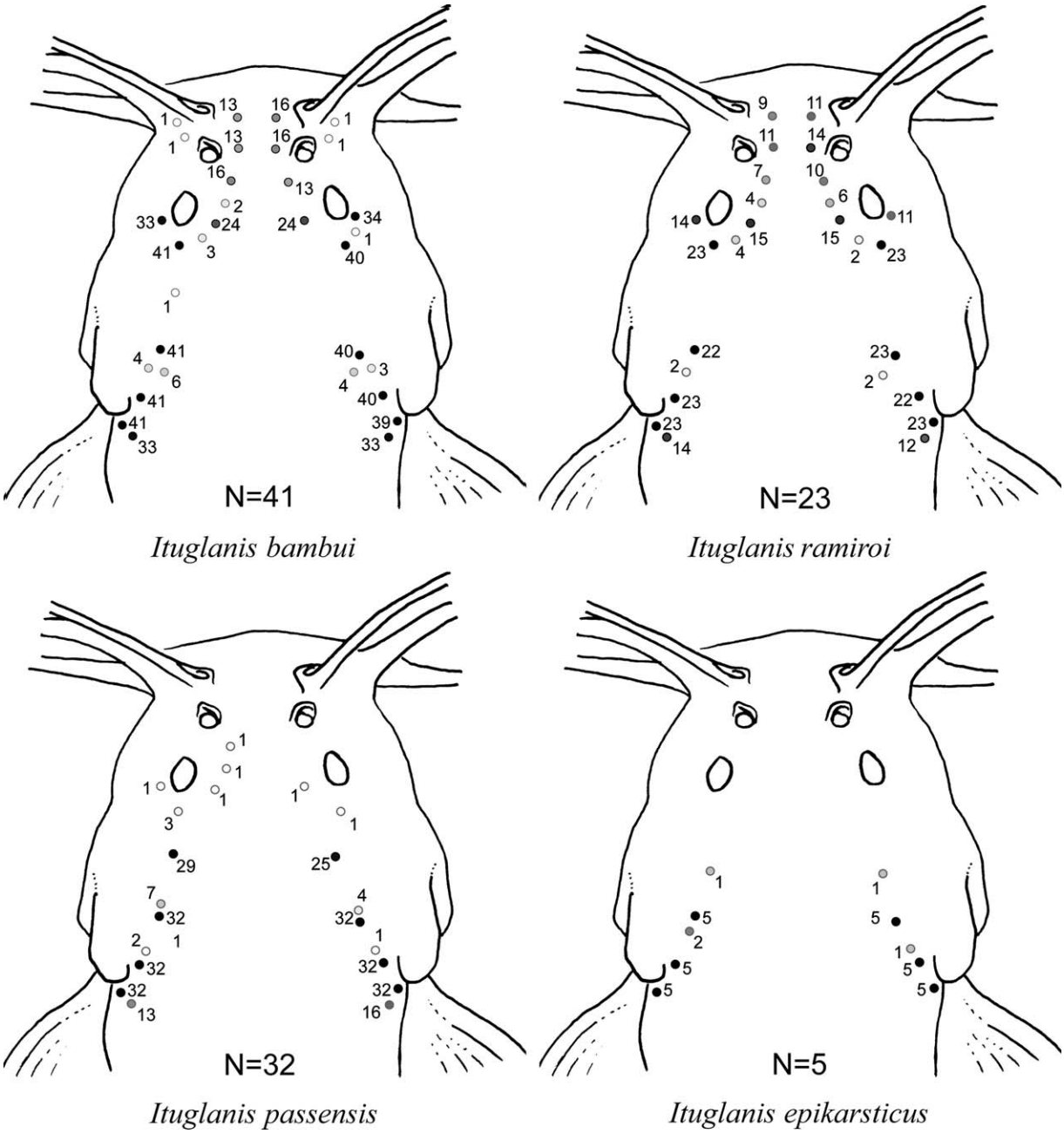

Fig. 9. Variability in the number of external openings of the laterosensory canals (i.e., pores) in the subterranean *Ituglanis bambui*, *I. ramiroi*, *I. passensis*, and *I. epikarsticus*. The number adjacent to each pore (dots) refer to the number of specimens in which each pore is present among the total number of specimens analyzed (*N*). The darker the pore, the higher its frequency among the specimens.

preoperculomandibular branch of the postotic canal (*sensu* Schaeffer and Aquino, 2000). The posterior half-pore opens at the dorsolateral contact between the pterotic and the posttemporo-supracleithrum, is fused to the anterior half-pore of the scapular canal, and represents the pterotic branch of the postotic canal (*sensu* Schaeffer and Aquino, 2000). Externally, the anterior pore opens dorsomedially to the opercular patch of odontodes, and the posterior pore opens approximately on the line passing through the posterior portion of the interopercular patch of odontodes.

The postotic laterosensory canal is present in all epigean and subterranean species of *Ituglanis* analyzed (Table 2).

Scapular Canal

The scapular canal bears two half-pores, therefore contains a single neuromast. It runs in an

anteroposterior direction at the posterolateral portion of the skull, its anterior portion enclosed by the dorsal portion of the posttemporo-supracleithrum (Figs. 3 and 4). Its posterior portion exits the skull at the contact between the posteroventral margin of the posttemporo-supracleithrum and the lateral tubular process of the Weberian capsule. It then extends caudally from this point, passing between the *epaxialis inferioris* and the *hypaxialis superioris* muscles, to the point where it encounters the trunk canal, forming a large pore. The anterior half-pore of the scapular canal is located at the contact between the pterotic and the posttemporo-spracleithrum, and the posterior half-pore is fused to the anterior half-pore of the trunk canal.

The scapular laterosensory canal is present in all epigean and subterranean species of *Ituglanis* analyzed (Table 2).

Trunk Canal

The trunk canal is present in all epigean species of *Ituglanis*, and in almost all of them, it has two half-pores, therefore containing a single neuromast. The canal runs in an anteroposterior direction at the horizontal septum, between the *epaxialis inferioris* and *hypaxialis superioris* muscles (Figs. 3 and 4). It is located dorsocaudal to the pectoral-fin base and caudal to the opercular patch of odontodes. The canal is reduced and restricted to a short tube, with its anterior half-pore fused to the caudalmost half-pore of the scapular canal, and its posterior half-pore caudal and very close to it, usually noticeably smaller than the anterior one and remaining as a half-pore. Because these fishes lack scales, only a small tubular bone may in some specimens enclose the trunk canal.

The trunk canal is slightly longer, with one additional neuromast and pore caudally, in *Ituglanis* sp. "Morena" (Canto, 2009), a clearly elongated species when compared to other *Ituglanis* species. This condition was also reported in a few specimens of *I. ina* (Wosiacki et al., 2012) and *I. boitata* (Ferrer et al., 2015) and we observed one specimen of *I. proops* with two additional pores caudally (Fig. 8). Among the subterranean species of *Ituglanis*, the trunk canal is present in most specimens of *I. boticario, I. mambai, Ituglanis* sp. "Posse", and *I. bambui*, and in about half of the specimens of *I. ramiroi* and *I. passensis* (Figs. 6 and 7). The trunk canal is absent in the more troglomorphic *I. epikarsticus* (Fig. 7).

VARIABILITY IN THE NUMBER OF CANAL PORES

We compared the intraspecific variability in the number of pores and number of canals and canal segments in all subterranean species of *Ituglanis* and in four epigean species: *I. australis, I. paraguassuensis, I. proops*, and *I. goya*. Figures 8 and 9 show the number of specimens where each pore is present. We considered the pattern of the laterosensory system for each species to be the pores and canals that are present on both sides of the head, with a frequency of at least 50% among the specimens analyzed.

The intraspecific variability on the laterosensory canal system in these species can be expressed in two ways. The first is variability in the presence of each canal or segment of canal, which results in the absence of the corresponding pores. Sometimes, the canal is present, but one of its pores is lacking; in these cases, the presence of the canal may be verified by viewing through a transparent trough the skin or by the presence of other pores associated with the canal. The second is the variability in the number of additional pores, which includes supranumerary pores and pores of the regular canals or segments of canals. These are symmetrically present with a frequency of 50% or less among the specimens analyzed, and thus do not define the pattern of the laterosensory system of the given species. These two aspects of the variability may combine in the different specimens, resulting in a wide variety of conditions for the laterosensory canal system.

Figure 10 shows the frequency of the presence of each canal or segment of canal—the frontal canal divided into anterior ("s3" to "s6") and posterior ("s6" to "i10") segments—among the specimens analyzed for the subterranean species of *Ituglanis*, and for the epigean *I. proops* for comparison. In the epigean *I. proops* (Fig. 10), as well as in *I. australis, I. paraguassuensis*, and *I. goya* the frequency of canals and pores is constant. In *I. proops*, for example, all canals and segments of canals were present with a frequency of almost 100%. In *I. goya*, the largest sample size analyzed (95 specimens), the frequency of presence of canals was at least 97%. In the subterranean species, however, the frequency is reduced, and the variability higher.

In *I. boticario* and *I. mambai*, the nasal, frontal (both anterior and posterior segments), otic, postotic, and scapular canals are present in all specimens analyzed (frequency of 100%), but the sphenotic and trunk canals, and especially the antorbital segment, are absent in some specimens. In *I. boticario*, the antorbital segment is symmetrically present with a frequency of more than 50%, but in *I. mambai* it is present in less than half of the specimens analyzed. In *Ituglanis* sp. "Posse", the sphenotic, otic, postotic, and scapular canals are present in all specimens, while the other canals may be absent in some specimens. The antorbital segment is present in only three specimens, and in one of these only in one side of the head.

For the remaining subterranean species, *I. bambui, I. ramiroi, I. passensis*, and *I. epikarsticus*,

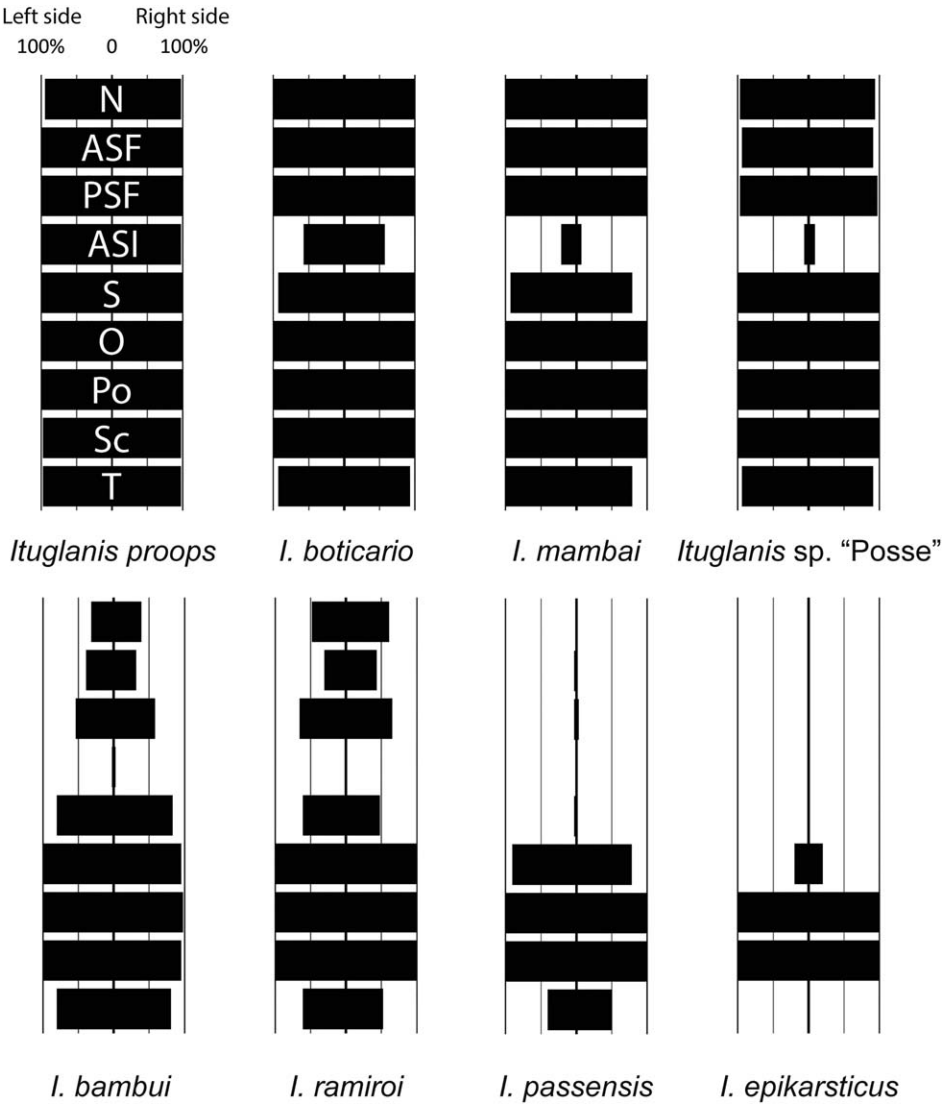

Fig. 10. Variability and asymmetry in the presence of canals and segments of canals in the epigean *Ituglanis proops* and in the subterranean species of *Ituglanis*. ASF = anterior segment of frontal. PSF = posterior segment of frontal. For other abbreviations, see Table 2.

the variability is even higher. Almost none of the canals or segments of canals have a frequency of 100% among the specimens analyzed. The antorbital segment of the infraorbital canal is absent in all specimens, except for one specimen of *I. bambui*. The canals with higher variability for these species are the nasal, frontal (both anterior and posterior segments), sphenotic, and trunk canals. In *I. passensis*, the sphenotic and the nasal canals are absent in all specimens, and the frontal canal is present in only one side of the head of two specimens. In *I. epikarsticus*, only the postotic and scapular canals are present, and the otic canal is present in only one side of the head of two specimens.

There is also a high asymmetry in the presence of canals and segments of canals among the specimens. For example, the trunk canal is absent on the right side of some specimens of *I. mambai*, while in the left side it is always present. In some cases, a given canal is present in more than half of the specimens in one side of the head, while in the other it is present in less than half, for example, the trunk canal in *I. passensis*, and the sphenotic and nasal canal in *I. ramiroi*.

Supranumerary pores are present in almost all species analyzed. More commonly, these supranumerary pores are present between the half-pores of the anterior or posterior segment of the frontal canal, or between the half-pores of the sphenotic, otic, and postotic canal. In *I. passensis*, the pores of the preoperculomandibular and the pterotic branch of the postotic canal are often branched, so that two pore openings are present

TABLE 3. Intraspecific variability of the laterosensory canal system in epigean and subterranean species of *Ituglanis*

	N	NPexp	TNPexp	–P	+P	Ivar	Fvar (%)
I. australis	16	20	320	19	0	19	6
I. paraguassuensis	21	24	504	25	0	25	5
I. proops	37	24	888	16	6	22	2
I. goya	95	18	1710	8	9	17	1
I. boticario	14	24	336	33	8	41	12
I. mambai	14	20	280	12	12	24	9
Ituglanis sp. "Posse"	33	20	660	33	34	67	10
I. bambui	41	14	574	64	106	170	30
I. ramiroi	23	12	276	38	117	155	56
I. passensis	32	8	256	10	53	63	25
I. epikarsticus	5	6	30	0	5	5	17

"*N*" = number of specimens analyzed. "NPexp" = number of pores expected for each adult specimen according to the pattern of laterosensory system for the species. "TNPexp" = total number of pores expected among all specimens of the species. "–P" = total number of pores lacking according to the expected. "+P" = total number of pores added to the expected. "Ivar" = instances of variability (= sum of –P and +P). "Fvar" = frequency of variability (ratio between Ivar and TNPexp).

instead of a single one. Although supranumerary pores are present in the specimens of almost all species analyzed, both epigean and hypogean, they are more frequent in the latter (Figs. 8 and 9). Because the hypogean species of *Ituglanis* are characterized by the reduction and absence of most of the canals that are present in other species of *Ituglanis*, when the supranumerary pores of these canals are present in some of these specimens they are considered additional pores. This means that they are additional to the pores that define the pattern of the laterosensory system of these species.

The variability in the presence or absence of canals or segment of canals among the specimens of a given species resulting in the absence of pores, combined with the presence of additional pores, which include the supranumerary, result in the high variability of number of pores in the laterosensory system observed. However, there is a difference in the degree of variability in the number of pores among epigean and subterranean species. The degree of variability is indicated by the frequency of variability ("Fvar," Table 3). This is the ratio between the number of instances of variability (i.e., pores that are lacking or added according to the laterosensory system pattern in each species) and the total number of pores expected for the specimens analyzed (calculated by the number of pores expected for each specimen according to the pattern of laterosensory system of each species).

Among the epigean species analyzed, the variability is 6% or less. In the epigean species *I. goya*, the largest sample size analyzed in the present study (n = 95), the variability is 1%. Among the subterranean species, however, the intraspecific variability is considerably higher. In the subterranean species from the Mambaí region, *I. mambai*, *I. boticario*, and *Ituglanis* sp. "Posse", the variability is 9%, 12%, and 10%, respectively, while in those from the São Domingos region, *I. epikarsticus, I. passensis*, *I. bambui*, and *I. ramiroi*, the variability is 17%, 25%, 30%, and 56%, respectively (Table 3).

DISCUSSION

In Siluriformes, according to Arratia and Huaquin (1995), the following laternal line canals are present: supraorbital (here treated as the nasal and frontal canals separately), infraorbital (here treated as composed of three portions: the antorbital, suborbital, and postorbital; the previous authors included also the sphenotic canal, here treated separately), otic, postotic, preopercular, mandibular, and the body lateral line (here treated as two different canals, the scapular and the trunk canal). In Trichomycterinae, the preopercular and mandibular canals are absent, the infraorbital is reduced to its antorbital segment, and the trunk canal is commonly very short, reduced to a few pores on its anterior region (Arratia and Huaquin, 1995).

The Laterosensory System Pattern in *Ituglanis*

The species of *Ituglanis* analyzed exhibit the general pattern of the laterosensory system described by Arratia and Huaquin (1995), but with considerable interspecific diversity, which may be useful both to diagnose species (Datovo and Landim, 2005; Sarmento-Soares et al., 2006; Lima et al., 2013; Datovo, 2014; Datovo and de Pinna, 2014; Rizzato and Bichuette, 2014; Ferrer et al., 2015; Datovo et al, 2016) and to give some clues about the phylogenetic relationships within the genus (see "Comments on *Ituglanis* phylogeny").

The nasal and frontal canals are usually continuous to each other and this condition is plesiomorphic among siluriforms and loricarioids according to Arratia and Huaquin (1995), being present in

Nematogenys, Copionodon, and *Glaphyropoma*, although in *Trichogenes*, the canals are separated from—and not even aligned to—each other. Among trichomycterines, the canals are also fused and continuous to each other in *Hatcheria* and *Bullockia* (Arratia and Huaquin, 1995), but are separate from each other in *Rhizosomichthys*. In *Eremophilus* and *Scleronema* the nasal canal is variably present (Arratia and Huaquin, 1995; DoNascimiento et al., 2014a). In *Silvinichthys*, the frontal canal is absent, and the nasal canal is present in *S. mendozensis, S. leoncitensis*, and *S. gualcamayo*, but is absent in *S. bortayro* and *S. huaichi* (Arratia, 1998; Fernández and de Pinna, 2005; Fernandez et al., 2011, 2013, 2014). In the more speciose genera of the subfamily, *Trichomycterus* and *Ituglanis*, the canals may be continuous or interrupted. In the three species of *Trichomycterus* recently suggested to be closely related to *Ituglanis* (DoNascimiento et al., 2014b), the nasal and frontal canals are continuous to each other, suggesting that this is the plesiomorphic condition for a clade composed of these three species plus *Ituglanis*. However, given the variable distribution of the character among trichomycterines in general and the lack of resolution about the phylogenetic interrelationships between trichomycterines, this hypothesis is now only tentative.

The presence of the infraorbital canal, represented by its antorbital portion only, is probably a plesiomorphic condition in Trichomycterinae, but the canal is absent in *Silvinichthys*, a genus whose species are characterized by several reductions in the laterosensory canal system, and at least in *Scleronema angustirostre* and one unidentified species of *Scleronema* analyzed by Arratia and Huaquin (1995). Among the three species of *Trichomycterus* recently suggested to be closely related to *Ituglanis* (DoNascimiento et al., 2014b), the infraorbital is present in *T. steindachneri* and *T. cachiraensis*, but is absent in *T. brachykechenos*. In *Ituglanis*, this antorbital segment is retained in a few epigean species: *I. agreste, I. australis, I. boitata, I. paraguassuensis*, and *I. proops* (Sarmento-Soares et al., 2006; Campos-Paiva and Costa, 2007; Lima et al., 2013; Datovo and de Pinna, 2014). In the remaining epigean species of the genus, the entire infraorbital canal is absent.

According to Allis (1889), the antorbital segment of the infraorbital canal is the first to be formed in *Amia*, and its formation is closely related to the formation of the nares. The author also remarks how adult specimens of *Amia* exhibit a highly developed laterosensory canal system when compared to other bony fishes (Webb, 1989), and how these different conditions of the laterosensory canals observed in other bony fishes could be compared to the different developmental stages of *Amia*'s laterosensory canal pattern. In fact, the condition of the laterosensory system in *Ituglanis* could be described as similar to a very early stage of *Amia*'s laterosensory system development, and the condition on the infraorbital canal is the most conspicuous of the examples to support this comparison. This is, that the only portion of the canal to develop is the antorbital portion, while the suborbital and postorbital portions, which in *Amia* develop later, are lacking. Interestingly, in *Nematogenys* and in the most basal subfamilies of Trichomycteridae—Trichogeninae and Copionodontinae—the infraorbital canal includes the antorbital portion and extends posteriorly to the point where it encounters the sphenotic canal (de Pinna, 1992; Arratia and Huaquin, 1995; Bichuette et al., 2008; Fig. 2 in de Pinna et al. 2010). However, in more derived trichomycterids (the entire Clade C, a well-supported monophyletic assemblage recovered frequently from the literature, e.g., de Pinna, 1998; Adriaens et al. 2010; Datovo and Bockmann, 2010; see Fig 1), the infraorbital canal, when present, is reduced to only its antorbital portion. The antorbital portion of the infraorbital canal is observed in some of the species of *Ituglanis* cited above, and the entire infraorbital canal is lacking in the remaining species. Therefore, the condition of the infraorbital canal observed in the more derived trichomycterids from Clade C could be interpreted as a developmentally truncated condition of the pattern observed in the more basal trichomycterids, as well as in *Nematogenys* and *Amia*.

The otic, postotic, and scapular canals form a continuous line of canals that is constant among trichomycterines. When reductions or absences of one or more of these canals occur, they involve mostly the otic canal only. For instance, in *Silvinichthys*, the otic canal is absent and the laterosensory system is comprised usually of the postotic and scapular canals, plus the nasal and a very short trunk canal (Arratia, 1998; Fernández and de Pinna, 2005; Fernandez et al., 2011, 2013, 2014). Within *Ituglanis*, reductions and absences occur only in the more troglomorphic subterranean species: *I. bambui* (otic absent in a few specimens), *I. ramiroi* (otic absent or reduced in some specimens), *I. passensis* (otic canal reduced in all specimens), and *I. epikarsticus* (otic usually absent). Elsewhere in Trichomycteridae, the otic canal is absent in some members of the clade TSVSG (Fig. 1, see below).

A complete trunk canal (Webb, 1989) is the plesiomorphic condition in Siluriformes (Arratia and Huaquin, 1995). In *Nematogenys*, the sister-group of Trichomycteridae (Fig. 1), the trunk canal is somewhat reduced and ends anteriorly to the dorsal-fin origin. Among trichomycterids, in copionodontines, the trunk canal is complete and reaches the caudal fin (except in the subterranean *Glaphyropoma spinosum*, see below), but in trichogenines, it is reduced, reaching the posterior

margin of the pectoral fin (de Pinna et al., 2010). The trunk canal is also reduced in trichomycterines. In *Hatcheria, Bullockia*, and some *Trichomycterus*, the trunk canal is a little longer, similar to *Trichogenes*, but in the remaining genera, including *Ituglanis*, it usually bears only two pores (Arratia and Huaquin, 1995). Canto (2009) described the *Ituglanis* sp. "Morena" as having three pores on the trunk canal, but this condition is probably related to the body elongation observed in this species. Additional pores posteriorly on the trunk canal were also observed in *I. ina* (Wosiacki et al., 2012), *I. boitata* (Ferrer et al., 2015), and *I. proops*.

The presence of a series of superficial neuromasts at the midline of the body, caudal to the trunk canal, as observed in *I. ina* by Wosiacki et al. (2012) is probably unique for the genus, but it is also present in *Silvinichthys mendozensis*. However, we agree with Wosiacki et al. (2012) that these conditions were more likely to have been acquired independently by the two taxa. This series of neuromasts present in *I. ina* and *S. mendozensis* probably represent the canal neuromasts that are primitively present in Siluriformes, but which in these species fail to enclose a canal forming a posterior continuation of the trunk canal (remaining as "replacement superficial neuromasts" sensu Webb, 2014). In support of this hypothesis, it may be noticed that in some taxa with a longer trunk canal, the canal may be variably interrupted (e.g., *Hatcheria*, *Trichogenes*, Arratia and Huaquin, 1995; de Pinna et al., 2010).

The Laterosensory Canal System in Subterranean Fishes

The subterranean species of *Ituglanis* exhibit more intraspecific variability in the laterosensory canal system than the epigean species, and as a result display a higher level of asymmetry, for both the presence of canals and segments of canals, and the number of additional pores. A higher variability on the laterosensory canal system has already been reported in other subterranean fishes. In *Bostrychus microphthalmus* (Perciformes: Gobiidae), Hoese and Kottelat (2005) reported high variability in the development of laterosensory canal pores. In *Rhamdiopsis krugi* (Siluriformes: Heptapteridae), Bockmann and Castro (2010) reported high variability in the number of pores and the patterning of canal ramifications. In subterranean populations of *Astyanax*, Wilkens (1988) reported high inter- and intra-individual variability caused by the fragmentation of the canals. Subterranean populations of *Poecilia mexicana* (Cyprinodontiformes: Poeciliidae) exhibit the cephalic laterosensory canal system variably fragmented, with the canal neuromasts sometimes exposed (Walters and Walters, 1965; Parzefall, 2001; Plath and Tobler, 2010). We also observed variability in the number and position of pores and in the presence of canals in *Trichomycterus itacarambiensis* and *T. dali* (Siluriformes: Trichomycteridae), with the variability being higher in the latter that is more troglomorphic. It is possible that a higher level of intraspecific variability in the laterosensory canal system is also present in other subterranean species, but this condition is rarely reported or described.

Another condition observed in the subterranean species of *Ituglanis*, when compared to epigean species, is a trend toward a reduction of the laterosensory canal system, with the loss of canals and/or segments of canals. This trend is more advanced in the species from the São Domingos karst area, which are considered more troglomorphic due to other characters such as reduction of eyes and pigmentation (Fernández and Bichuette, 2002; Bichuette and Trajano,2004, 2008; Rizzato and Bichuette, 2014). This suggests that the degree of reduction of the laterosensory canal system could be related to the degree of specialization to the subterranean habitat.

In the subterranean habitat, the laterosensory system is one of the main sensory systems that can be used by fish (and also amphibians) in the absence of light (Montgomery et al., 2001; Dezfuli et al., 2009; Niemiller and Poulson, 2010; Windsor et al., 2008), and some information about the degree of development of this system is commonly cited in the literature (see below). The expectation, considering the general phenomena of sensory compensation usually observed in subterranean organisms (Barr, 1968; Langecker, 2000; Montgomery et al., 2001; Culver and White, 2005; Dezfuli et al., 2009), is that the laterosensory system would be somehow hyperdeveloped in subterranean fishes given its importance as a substitute for the light-related sensory systems, which in the permanently aphotic conditions of subterranean environments become useless.

However, most subterranean fishes are described as having the laterosensory canal system reduced, fragmented, or even lacking. For example, Berti and Messana (2010) report a reduction of the lateral line on the body of *Phreatichthys andruzzii* (Cypriniformes: Cyprinidae), with many interruptions along the canal. Chen et al. (2010) report fewer lateral line scales with consequent reduction of the trunk canal in subterranean species of *Sinocyclocheilus* (Cypriniformes: Cyprinidae). In *Protocobitis typhlops* (Cypriniformes: Cobitidae), the entire laterosensory canal system is absent (Yang et al., 1994). In *Bostrychus microphthalmus* (Perciformes: Gobiidae), the cephalic and trunk canals are reduced (Hoese and Kottelat, 2005). Lundberg (1982) considered the reduction of the trunk canal an adaptation of the subterranean species of Ictaluridae (Siluriformes),

shared by *Trogloglanis pattersoni, Satan eurystomus, Prietella phreatophila*, and *P. lundbergi*. Trajano and Bichuette (2010) list some Brazilian subterranean fishes with reduction of trunk canals: *Stigichthys typhlops* (Characiformes: Characidae), *Rhamdiopsis krugi*, and species of *Phreatobius* (Siluriformes: Phreatobiidae). Other Brazilian subterranean species with reduction of trunk canals are *Glaphyropoma spinosum* (the unique subterranean copionodontine described so far and unique in that the trunk canal is reduced), *Trichomycterus itacarambiensis, T. dali*, and, of course, the subterranean *Ituglanis*. Actually, in these two species of *Trichomycterus* and in the subterranean species of *Ituglanis*, the canals are greatly reduced in the more troglomorphic species, suggesting that the degree of canal reduction is somehow related to the overall degree of specialization to the subterranean environment. The examples given above suggest, contrary to expected, that the laterosensory canal system of subterranean fishes is reduced in comparison with their epigean relatives, arguing against mechanisms of sensory compensation for this sensory system as a whole.

However, most authors do not make a clear distinction between the laterosensory submodality of canals and of superficial neuromasts when describing the modifications observed in subterranean species. This distinction is importance because is important because it has been demonstrated that these subsystems not only have developmental and morphological differences (Webb, 2014), but also perform different functions (Kroese and Schellart, 1992; Engelmann et al., 2000; Montgomery et al., 2001; Engelmann et al., 2002; McHenry and Liao, 2014; Netten and McHenry; 2014; Chagnaud and Coombs, 2014; Montgomery et al., 2014). Canal neuromasts detect accelerations and superficial neuromast detect velocity, and canal neuromasts respond to higher frequencies than superficial neuromasts, in a variety of behavioral contexts McHenry and Liao, 2014; van Netten and McHenry, 2014). Therefore, these submodalities have different roles according to the environmental demands on the individuals.

Additionally, when canals do not form, the canal neuromasts often remain exposed on the skin and may even proliferate (Webb, 2014). In fact, Langecker and Longley (1993) found larger canals and pores of the laterosensory system in the subterranean *Satan eurystomus* and *Trogloglanis pattersoni* (Siluriformes: Ictaluridae), and the canals were usually interrupted and fragmented. However, they argue that the fragmentation and reduction on the laterosensory canals in these fishes are compensated by a more developed or exposed system of superficial neuromasts. The hyperdevelopment of the superficial neuromasts may be accomplished by their proliferation or by modifications of these organs that would result in enhanced ability to detect and react to stimuli.

Montgomery et al. (2001) state that there is no difference in the number of superficial neuromasts in the majority of subterranean fish species when compared with their epigean relatives, but they did not mention which species they were discussing. In contrast, Teyke (1990) demonstrated that the cupulae of superficial neuromasts are longer in subterranean *Astyanax* from México than in epigean species, and Poulson (1963) demonstrated that the exclusively subterranean species of Amblyopsidae have hyperdeveloped superficial neuromasts aligned in crests, while the canals are reduced in the head and absent in the body (Niemiller and Poulson, 2010). Therefore, it is possible that, even without an increase in the number of superficial neuromasts, structural modifications of these organs, as observed in the subterranean *Astyanax* and amblyopsids, may be responsible for an increase in the sensitiveness of the laterosensory system as a whole. This would make it a good substitute for the light-related sensory systems in the subterranean environment (Teyke, 1990). Indeed, Yoshizawa et al. (2010) demonstrated that the subsystem of superficial neuromasts specifically is involved in better perception of small vibrating objects in troglomorphic individuals of *Astyanax*, increasing their efficiency to find food.

This trend toward reduction of the laterosensory canal system and hyperdevelopment of superficial neuromasts, which appears to be universal for subterranean fishes, would be advantageous in slower, less turbulent water environments (Dijkgraaf, 1962; Langecker and Longley, 1993), or for more sedentary, slow swimming fishes (Engelmann et al., 2000, 2002). Interestingly, in species living in non-subterranean but confined spaces, like clefts and crevices, or burrowed in leaf litter, rocks, sand, or interstitial environments, similar patterns in relation to the laterosensory system are found. For example, in *Typhlogobius californiensis* (Perciformes: Gobiidae), the canals are reduced and fragmented, and there is variability in the patterning of pores (Ahnelt and Scattolin, 2003).

The family Trichomycteridae itself, as a whole, is a good example of this relationship between the reduction of the laterosensory canals and a more secretive, cryptobiotic habitat. Trichomycterids exhibit a large diversity of patterns in the laterosensory system, and the reduction and loss of canals or segments of canals is a general trend, as the more derived lineages lack the canals present in the basal members of the group (de Pinna, 1989a; Arratia and Huaquin, 1995). The main reductions involve the infraorbital, nasal, frontal, and trunk canals (Arratia and Huaquin, 1995; Sarmento-Soares et al., 2006). In some taxa, reductions of the otic and postotic canals also

occur, for example, in *Silvinichthys* (Arratia and Huaquin, 1995; Arratia, 1998; Fernández and de Pinna, 2005) and in the glanapterygines *Listrura* (except for *L. tetraradiata), Typhlobelus*, and *Pygidianops magoi* (de Pinna, 1989b; Arratia and Huaquin, 1995; Landim and Costa, 2002; de Pinna and Wosiacki, 2003; de Pinna and Zuanon, 2013). The reduction of the laterosensory system of canals is considered a synapomorphy of Glanapteryginae (Baskin, 1973; de Pinna, 1988, 1989b; Schaeffer et al., 2005), and the entire canal system is lacking in *Typhlobelus auriculatus* (de Pinna and Zuanon, 2013).

Interestingly, the taxa with more reduced laterosensory canals among trichomycterids are those with cryptobiotic habits, living under rocks (*Silvinichthys*, Arratia 1998); among leaf litter, sand, and gravel (*Sarcoglanis* and *Malacoglanis*, Myers and Weitzmann, 1966; *Listrura* and *Glanapteryx*, Schaeffer et al., 2005; de Pinna and Wosiacki, 2003); in phreatic (*Silvinichthys bortayro*, Fernández and de Pinna, 2005), interstitial, or psammophilic environments (*Pygidianops* and *Typhlobelus*, de Pinna and Wosiacki, 2003; Schaeffer et al., 2005; de Pinna and Zuanon, 2013); and, of course, in subterranean habitats (the subterranean *Ituglanis* and *Trichomycterus*). Even clade C of Trichomycteridae, which includes Trichomycterinae and other more derived subfamilies of the "TSVSG" clade (de Pinna, 1992, 1998; Bockmann et al., 2004; Datovo and Bockmann, 2010, see Fig. 1), whose species utilize more hidden habitats on rocks, leaf litter, or even burrowed in the substrate, exhibit reductions of the laterosensory canals system (e.g., the absence of the posterior portions of the trunk and infraorbital canals; de Pinna, 1992; Arratia and Huaquin, 1995; Datovo and Bockmann, 2010). This contrasts with the basal-most subfamilies Trichogeninae and Copionodontinae, whose species explore more frequently the water column (de Pinna and Wosiacki, 2003; Sazima, 2004; de Pinna et al., 2010; Zanata and Primitivo, 2013).

Therefore, it may be concluded that reduction in the canals of the laterosensory system, which may be accompanied by a proliferation of superficial neuromasts, appears to be correlated not only with the subterranean habitat specifically, but with other instances of secretive habits in confined spaces. When present in subterranean species, it may be regarded as a troglomorphic character, and its use for inferences about the relationships among species including subterranean lineages should be used cautiously, as they may represent convergences (i.e., homoplasies) for those different lineages that independently colonized the subterranean environment (see below).

Finally, it is noteworthy that in glanapterygines, especially the more derived, interstitial-living *Pygidianops* and *Typhlobelus*, the extreme reduction of the laterosensory canal system is accompanied by the reduction or absence of eyes and pigmentation (Schaeffer et al., 2005; de Pinna and Zuanon, 2013), which are two other characters commonly related to life in the subterranean environment (= troglomorphic characters).

Comments on *Ituglanis* Phylogeny

To date, the relationships within *Ituglanis* are still unresolved, although many authors discuss possible relationships among some species and the existence of clades supported by the sharing of some morphological characters (de Pinna and Keith, 2003; Sarmento-Soares et al., 2006; Bichuette and Trajano, 2008; Wosiacki et al., 2012; Lima et al., 2013; Datovo, 2014; Rizzato and Bichuette, 2014; Datovo et al., 2016). Recently, DoNascimiento et al. (2014b) provided evidence of a close relationship between three species of *Trichomycterus: T. brachykechenos, T. cachiraensis*, and *T. steindachneri*, and the species of *Ituglanis*, based on a supposed morphocline in relation to the reduction of the cranial fontanel. This suggestion provided a putative outgroup to which the species of *Ituglanis* can be compared to determine the direction of changes inside the group.

The first attempt to identify monophyletic groups within the genus was made by de Pinna and Keith (2003). In that work, the authors suggested the existence of at least one clade inside the genus uniting those species with an apomorphic reduction in the number of ribs to three or less pairs. Nowadays, that clade would include species occurring in the northwestern South American river basins, comprising the Amazon basin and tributaries, including the Araguaia (*I. macunaima*) and the lower Tocantins (*I. ina*) River basins, rivers in Colombia (*I. guayaberensis* and *I. metae*), in the Guyanas (*I. gracilior* and *I. nebulosus*), and also the Paraná-Paraguay River basin (*I. eichorniarum* and *I. herberti*) (see Table 2). The remaining species, which have four or more pairs of ribs (a plesiomorphic condition as basal trichomycterids, including *T. brachykechenos, T. cachiraensis*, and *T. steindachneri*, have nine or more pairs of ribs), occur in southeastern South America, mostly in coastal river basins, but also in the upper Tocantins River basin (*I goya* and the subterranean species; Rizzato and Bichuette, 2014). The only exception to this scheme is *I. laticeps*, which was described from Ecuador, thus being geographically closest to the northwestern group, but have four pairs of ribs, which approximates it to the southeastern group. Nevertheless, it may be assumed that the geographical distribution of the species provides, at least, partial support for this scheme.

In support of this scheme, Lima et al. (2013) suggested that the genus *Ituglanis* would be an

example of a distribution pattern described by Ribeiro (2006) as the "biogeographic pattern B." This is where the species of coastal drainages of Brazil are closely related to each other and form a clade that is a sister group to another clade that spreads in the Brazilian shield and the Amazon basin. Although this description is an imprecise simplification of the biogeography of *Ituglanis* suggested by de Pinna and Keith (2003), the members of the more derived clade are distributed in the northwestern basins of South America, mainly in the Amazon basin and its tributaries, while the remaining members of the genus commonly occur in southeastern Brazilian coastal drainages.

De Pinna and Keith (2003) also proposed two smaller clades, one supported by the presence of a widening of the mesethmoid shaft, and the other supported by the presence of a posterolateral process on the "antorbital" (actually the sesamoid supraorbital, see Datovo and de Pinna, 2014: Fig. 4). However, we noticed that these characters might vary within a species, and suggest a broader intra- and interspecific analysis to evaluate the usability of these characters for recognizing clades inside *Ituglanis*.

Rizzato and Bichuette (2014) proposed that the absence of the anterior (= antorbital) portion of the infraorbital may be an apomorphic condition shared by the more derived species of *Ituglanis*. That clade would include at least *I. cahyensis*, *I. parahybae*, and *I. goya* from the southeastern group, and the remaining species from the northwestern clade of de Pinna and Keith (2003; Table 2). In this clade, *I. cahyensis* and *I. parahybae*, both from coastal drainages in southeastern Brazil, may be grouped by the loss of the nasal canal, and also by the low number of pectoral-fin rays, five rays (one unbranched plus four branched), and by coloration formed by three longitudinal stripes (Sarmento-Soares et al., 2006). Although *I. macunaima* also lacks the nasal canal and has five pectoral fin rays (Datovo and Landim, 2005), it is more parsimonious to interpret these similarities as homoplastic, given that this species is from the clade with three or less pairs of ribs, while *I. cahyensis* and *I. parahybae* have four and six pairs of ribs, respectively. The absence of the nasal canal and the pigmentation forming longitudinal stripes also occur in *I. australis*, but again this must be interpreted as an independent loss, as *I. australis* presents the antorbital segment of the infraorbital and can be differentiated from *I. cahyensis* and *I. parahybae* by several morphological characters (Datovo and de Pinna, 2014).

The remaining species of *Ituglanis*, which retained the plesiomorphic conditions of having four or more pairs of ribs plus the antorbital portion of the infraorbital canal, include *I. agreste, I. australis, I. boitata, I. paraguassuensis, I. proops*, and the subterranean species. At this point, it becomes clear why the inclusion of troglomorphic characters in phylogenetic analysis, specifically those related to the laterosensory system, must be made with caution. As discussed above, the reduction in the laterosensory system of canals is clearly related to the specialization for living in a subterranean environment. As absences of canals are expected to occur in subterranean lineages, the mere inclusion of these characters on a phylogenetic matrix without considering this may result in the homoplastic grouping of non-related species. For example, the absence of the antorbital, nasal, and frontal canals would group *I. epikarsticus* and *I. passensis* with *I. parahybae*, although this grouping would conflict with the geographical distribution of these species. Indeed, the geographical distribution of the subterranean species, and many other morphological similarities, suggests that they are more closely related to each other than to any other epigean species of *Ituglanis*. Thus, it is more parsimonious to assume that the four subterranean species of *Ituglanis* from the São Domingos karst area, *I. ramiroi, I. bambui*, *I. passensis*, and *I. epikarsticus*, are more closely related to their geographically closest relatives, the subterranean species from the Mambaí karst area. This would mean that the reduction or absence of the antorbital, nasal, and frontal canals in these species occurred independently as a result of specialization to the subterranean environment.

Inside the paraphyletic group that includes the more plesiomorphic species of *Ituglanis*, another subgroup could be hypothesized, reuniting the species that share an interruption between the nasal and the frontal canals: *I. boitata, I. proops*, and the subterranean species. The presence of this interruption is probably apomorphic for *Ituglanis*, as the nasal and frontal canals are continuous to each other in the more basal Siluriformes, in *Nematogenys*, and in most of the basal trichomycterids and trichomycterines, including *T. brachykechenos, T. cachiraensis*, and *T. steindachneri* (see "The laterosensory system pattern in *Ituglanis*" above). Again, because the subterranean species of São Domingos usually lack the nasal and frontal canals, making this character inapplicable, the inclusion of these species in this subgroup is justified by their close proximity with the subterranean species of Mambaí, which suggests that their ancestors also exhibited the interruption between the nasal and frontal canals. In support of this, it must be noticed that when the nasal and frontal canals are present in some specimens of *I. bambui* and *I. ramiroi*, they are always separated from each other (i.e., interrupted).

The situation of *I. agreste* and *I. australis* is somewhat problematic. The latter lacks the nasal canal, a loss that is probably homoplastic with the one in *I. macunaima* and the one in *I. cahyensis* plus *I. parahybae*. However, the absence of the

nasal canal makes it impossible to determine if in the ancestral condition *I. australis* had the nasal and frontal canals fused or interrupted. In the case of *I. agreste*, if it is assumed that the plesiomorphic condition is of fusion between the nasal and frontal canals, we should consider that in this species the entire canal formed by the fusion of the nasal and frontal canals is shortened (the opinion followed here). However, if it is assumed that the plesiomorphic condition is the interruption between these canals, we should consider that in this species the nasal canal is posteriorly displaced and secondarily fused to the frontal. This would mean then *I. agreste* would be included in the subgroup containing the species with nasal and frontal canals interrupted: *I. proops*, *I. paraguassuensis*, and the subterranean species.

Finally, although the discussion above provides evidence that the pattern of the laterosensory system can be useful to recognize relationships among species of *Ituglanis*, the hypotheses proposed above are premature, and consider only the number of ribs and the patterning of the laterosensory systems, coupled with some biogeographical information, to infer the existence of groups of species within the genus. We agree with Datovo and Landim (2005), Sarmento-Soares et al. (2016), and Datovo and de Pinna (2014), and Datovo et al. (2016) that a more definitive proposal of relationships among species of *Ituglanis* will only be achieved as the result of phylogenetic analysis supported by a broad sampling of both taxa and characters. However, we believe that our findings provide a framework of preliminary hypotheses that may represent a first step forward to unraveling the phylogenetic history of this particular taxon of the astonishingly diverse family Trichomycteridae.

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