Morphological and biochemical comparison of two allopatrid populations of *Hypostomus margaritifer* (Regan, 1907) (Osteichthyes, Loricariidae) from the upper Paraná River basin, Brazil

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ABSTRACT. Two allopatrid populations of armoured catfishes, both referred to as *Hypostomus margaritifer* (Regan, 1907) (Osteichthyes, Loricariidae), were collected in the Corumbá reservoir (State of Goiás) and in the Itaipu reservoir (State of Paraná), Brazil. Differences in colour pattern, arrangement of abdominal platelets and maximum length of specimens were observed between these populations. Their taxonomic status was investigated through two different approaches: morphological and allozyme analysis. Morphometrics showed no expressive differences between these two populations and the holotype of *H. margaritifer*. A unique difference in allelic frequencies was observed in the sAat-A locus; nevertheless conspecificity of the two populations was confirmed by a high identity value (0.988). A complementary description of *H. margaritifer* is given as well as a discussion of the most important studies.

Key words: *Hypostomus margaritifer,* allozymes, Loricariidae, morphometrics, Paraná River, systematic.

RESUMO. Comparação morfológica e bioquímica de duas populações alopátridas de *Hypostomus margaritifer* (Regan, 1907) (Osteichthyes, Loricariidae) da bacia do alto rio Paraná. Duas populações alopátridas de cascudos, ambas provisoriamente denominadas *Hypostomus margaritifer* (Regan, 1907) (Osteichthyes, Loricariidae), foram coletadas no reservatório de Corumbá, Estado de Goiás e no reservatório de Itaipu, Estado do Paraná. Diferenças no padrão de coloração, presença ou ausência de placas abdominais e no tamanho máximo dos exemplares foram observadas entre estas populações. O status taxonômico dessas populações foi investigado através de duas técnicas diferentes: análise morfológica e de aloenzimas. A análise morfométrica não permitiu detectar diferenças expressivas entre as populações. Uma única diferença significativa nas freqüências alélicas foi encontrada no loco sAat-A, entretanto a conspecificidade é comprovada pelo alto valor de identidade genética (0,988). Uma descrição complementar de *H. margaritifer* é dada, assim como uma discussão dos trabalhos mais importantes.

Palavras-chaves: *Hypostomus margaritifer*, aloenzimas, Loricariidae, morfometria, rio Paraná, sistemática.

Introduction

Since the description of *Plecostomus* (= *Hypostomus*) *margaritifer* from the Piracicaba River basin, upper Paraná River basin (above Itaipu dam), by Regan in 1907, few studies have been found in the literature dealing with this species. Systematics of *Hypostomus* species, from the upper Paraná River basin is still poorly known, mainly because most of the 19th and early 20th century descriptions are unfinished, which makes the recognition of most species occurring in this system difficult. The knowledge of the intraspecific variability in morphology and color pattern, related to the distribution and relationships of the species of the genus *Hypostomus*, is even nowadays really unknown. Joining classic and molecular approaches in systematics, such a gap of information could be filled (Zawadzki *et al*., 1999).

Recent collections in the Itaipu Reservoir (Figure 1) made the recognition of a *Hypostomus* population possible, which seems to fall into the range of Regan's description of *H. margaritifer*. At the same time, an ichthyologic survey in the Corumbá Reservoir (Figure 1), an affluent of the Paranaíba River, also in the upper Paraná River basin, showed a very similar population.

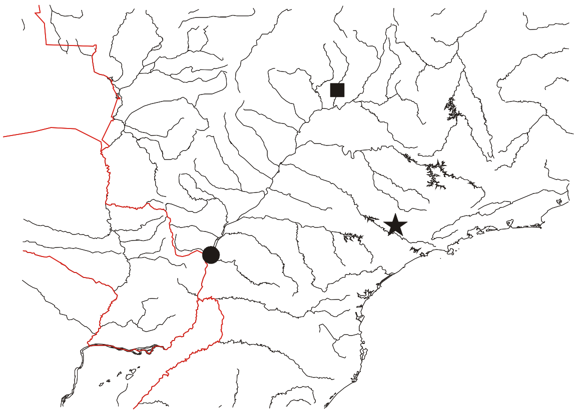

Figure 1. Southern portion of South America showing the type-locality of *Hypostomus margaritifer* (Regan, 1907) (star) and the collection sites of populations from the Itaipu (circle) and Corumbá reservoirs (square)

In order to quantify the differences and similarities between the two allopatrid populations and clarify their specific status, we recorded morphometrics and counts from both populations and compared them with the holotype of *H. margaritifer* from the Piracicaba River. We also sampled fresh tissues to perform allozyme electrophoresis. In this way, we intend to question geographical, genetic and morphological variability for this taxon in order to establish the correct taxonomic status for these populations.

Material and methods

Specimens examined

The material is deposited in the ichthyologic collection of Nupélia - Núcleo de Pesquisas em Limnologia, Ictiologia Aquilcultura (NUP). Voucher specimens are at Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Porto Alegre, Brazil (MCP 26671). The holotype of *H. margaritifer*, from the Piracicaba River, deposited at the Natural History Museum (BMNH), was also examined for comparative purposes.

BMNH 1907.7.6.14; holotype; 125.2 mm SL; Piracicaba River, state of São Paulo; collector R. v. Ihering.
NUP 1438; 10 ex. 227.0 - 312.0 mm SL; Itaipu reservoir, Guaíra town, State of Paraná; 25°14'12"S/54°14'02"W; 15.iv.1997; collector Nupélia.
NUP 1439; 12 ex. 131.3 - 184.2 mm SL; Corumbá Reservoir, between Pires do Rio and Caldas Novas, state of Goiás; 17°29' - 17°51'S/48°22' - 48°33'W; 17.vii.1996 - 09.xii.1999; collector Nupélia.
NUP 1573; 8 ex. 139.0 - 232.0 mm SL; the same locality as above; 27.x.1997 - 10.xii.1999; collector Nupélia.

Morphological analyses: Twenty specimens from the Itaipu Reservoir and twenty from the Corumbá Reservoir were analysed. Measurements were made with a Mitutoyo Digimatic calliper. Measurements and counts were taken as described by Boeseman (1968) and Weber (1985).

Electrophoretic analyses: White skeletal muscle, liver and heart tissues from 15 live specimens from each reservoir were sampled and stored in liquid nitrogen until analysis. Electrophoretic procedures are detailed in Zawadzki *et al.* (1999). Fourteen enzymatic systems were analysed (Table 1). The nomenclature used was proposed by the International Union of Biochemistry and Molecular Biology (1992). Data were analysed using Biosys 1 software (Swofford and Selander, 1981). The genetic interpretation of the enzymatic patterns was based on the quaternary structure of enzymes described by Ward *et al*. (1992).

Results

Morphological complementary description of *Hypostomus margaritifer* (Regan, 1907).

Morphometric data and counts are showed in Table 2.

Head completely covered with small, juxtaposed dermal ossification, excluding a small naked area on tip of snout. This last obtuse, narrowed anteriorly. Dorsal margin of orbit elevated, continuing in a low ridge on post-temporal region. Temporal plates not keeled. Supraoccipital and predorsal regions with a weak median ridge. Supraoccipital bone without remarkable posterior process and bordered by a single plate.

Mouth medium sized; maxillary barbells approximately twice orbital diameter. Teeth strong with elongated crown and small lateral cuspid. Mandibulary teeth forming an angle of approximately 80°. Outer edge of upper lips covered with small scutelets, inner surface of lower lips covered with numerous little papillae.

Table 1. Names, enzyme commission number(E. C. no.), tissues, buffers, quaternary structure (Q.S.) and number of loci of each enzyme of *Hypostomus margaritifer* (Regan, 1907) from the Itaipu and Corumbá reservoirs. L = liver; M = muscle; H = Heart; TBE = Tris/borate/EDTA (pH 8.7) (Boyer *et al.*, 1963); TC = Tris/citrate (pH 7.0) (Shaw and Prasad, 1970)

Enzyme (Abbreviation)	E.C. no.	Tissue	Buffer	Q. S.	Loci
Acid phosphatase (Acp)	3.1.3.2	L	TC	Dimeric	1
Alcohol dehydrogenase (Adh)	1.1.1.1	L	TBE	Dimeric	1
Aspartate transaminase (Ata)	2.6.1.1	L, H, M	TBE	Dimeric	2
Glucose 1-dehydrogenase - NAD+ (Gcdh)	1.1.1.118	L	TBE	Dimeric	1
Glycerol-3-phosphate dehydrogenase (G3pdh)	1.1.1.8	L, H, M	TC	Dimeric	2
Glucose-6-phosphate dehydrogenase (G6pdh)	1.1.1.49	L	TBE	Tetrameric	2
Glucose-6-phosphate isomerase (Gpi)	5.3.1.9	L, H, M	TC	Dimeric	2
Isocitrate dehydrogenase - NADP+ (Icdh)	1.1.1.42	L, H, M	TC	Dimeric	2
L-Lactate dehydrogenase (Ldh)	1.1.1.27	H, M	TC	Tetrameric	2
Malate dehydrogenase (Mdh)	1.1.1.37	L, H, M	TC	Dimeric	3
Malate dehydrogenase - NADP+ (Mdhp)	1.1.1.40	L, H, M	TC	Tetrameric	2
Phosphoglucomutase (Pgm)	5.4.2.2	L, H, M	TC	Monomeric	1
Peroxidase (Per)	1.11.1.6	L, H	TC	Tetrameric	3
Superoxide dismutase (Sod)	1.15.1.1	L, H, M	TBE	Dimeric	1

Table 2. Morphometric data and counts of holotype and populations from the Itaipu and Corumbá reservoirs of *Hypostomus margaritifer* (Regan, 1907). Nl: Nostril length; Iow: Inter-orbital width; Pdd: Pre-dorsal distance; Pacpl: Post-anal caudal peduncle length. * Data not available

Character	Itaipu			Corumbá			Piracicaba
	N	Range	Mean	N	Range	Mean	Holotype
Standard length (mm)	20	227.0-312.0	275.8	20	124.8-232.0	162.1	125.2
Proportions of standard length							
Pre-dorsal distance	20	2.4-2.7	2.5	20	2.3-2.6	2.4	2.4
Head length	20	3.0-3.4	3.2	20	2.9-3.3	3.0	2.9
Cleitral width	20	3.0-3.5	3.2	20	3.1-3.4	3.2	3.1
Head height	20	4.8-5.7	5.2	20	4.9-5.5	5.2	5.5
Dorsal base length	20	3.4-4.2	3.8	20	3.4-4.1	3.8	3.5
Inter-dorsal length	20	4.8-6.2	5.5	20	5.0-6.6	5.9	6.1
Post-anal caudal peduncle length	20	3.0-3.5	3.2	20	2.9-3.6	3.2	3.7
Caudal peduncle height	20	8.2-9.2	8.7	20	8.2-9.7	8.9	8.7
Dorsal ray length	20	2.3-3.1	2.6	20	2.7-3.4	3.0	2.7
Pectoral ray length	20	2.6-3.1	2.9	20	2.9-3.5	3.2	3.1
Pelvic ray length	20	3.4-3.9	3.6	20	3.4-4.2	3.7	3.5
Thoracic length	19	3.6-4.5	4.2	20	3.6-4.9	4.2	3.6
Abdominal length	20	3.9-4.5	4.0	20	3.8-4.4	4.1	5.4
Superior caudal ray length	18	2.1-3.3	2.9	20	2.7-3.8	3.2	*
Inferior caudal ray length	19	2.1-3.8	2.8	20	2.6-3.4	2.8	2.7
Adipose length	18	9.4-12.1	11.0	20	9.1-12.5	10.6	10.9
Proportions of head length							
Cleitral width	20	1.0-1.1	1.0	20	1.0-1.1	1.1	1.0
Head height	20	1.5-1.8	1.6	20	1.6-1.9	1.7	1.9
Nostril length	20	1.4-1.5	1.5	20	1.5-1.6	1.6	1.6
Orbital diameter	20	5.6-7.0	6.3	20	4.2-6.2	5.0	5.1
Inter-orbital width	20	2.4-3.0	2.7	20	2.5-3.0	2.8	2.9
Barbel length	20	8.1-10.8	9.1	20	5.2-8.2	6.6	15.8
Mandibular length	20	5.1-6.7	5.7	20	5.1-7.1	5.9	5.4
Some usual corporal measures							
Nl/Orbital diameter	20	3.6-4.7	4.3	20	2.7-4.0	3.2	3.2
Iow/Orbital diameter	20	1.7-2.7	2.1	20	1.4-2.4	1.8	1.9
Iow/Mandibular length	20	1.8-2.8	2.4	20	1.8-2.8	2.1	1.8
Pdd/Dorsal ray length	20	0.9-1.2	1.0	20	1.1-1.4	1.2	1.1
Pacpl/Caudal peduncle height	20	2.4-2.9	2.7	20	2.4-3.1	2.7	2.4
Counts							
Lateral plates	20	26-27	26.5	20	26-27	26.3	27
Pre-dorsal plates	20	3	3.0	20	3	3.0	*
Supra-occipital base plates	20	1	1.0	20	1	1.0	1
Dorsal-adipose plates	20	6-7	6.7	20	5-7	6.1	*
Adipose-caudal plates	20	5-7	6.2	20	5-7	6.0	*
Anal-caudal plates	20	14-15	14.7	20	14-15	14.7	*
Dorsal base plates	20	8	8.0	20	8-9	8.1	*
Anal base plates	20	2-3	2.1	20	2	2.0	*
Dentary teeth	20	19-32	24.8	20	16-24	20.8	21
Maxillary teeth	20	19-32	25.8	20	19-31	22.4	18

Body width in cleithral region greater than depth; dorsal profile rising slightly from tip of snout to dorsal fin origin, and decreasing from this point to end of caudal peduncle. Body covered with five rows of not keeled scutes along body sides. Caudal peduncle elliptic in cross-section slightly flattened ventrally. Lateral line complete. Abdomen and lower surface of head covered with minute scutelets. General aspects of body shape shown in lateral, dorsal and ventral views in Figure 2.

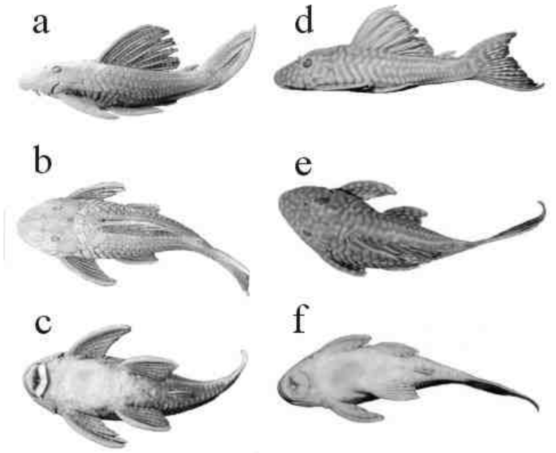

Figure 2. *Hypostomus margaritifer* (Regan, 1907) from the Itaipu Reservoir, 272.0 mm SL (a, b, c) and from the Corumbá Reservoir, 184.0mm SL (d, e, f)

Dorsal fin large, when depressed, reaching adipose fin. Pectoral fin ray covered with denticles distally elongated in larger specimens. Ventral fin rays flattened ventrally. Adipose fin with a ray generally not curved. Caudal fin strongly forked, with inferior lobe slightly longer than the superior one.

Differences between populations:

Itaipu specimens usually present a smaller orbital size, with the first dorsal fin ray proportionally longer and reaching the adipose fin, a fact which generally does not occur in Corumbá specimens. Adult specimens from Itaipu present a much larger size, with the abdomen always totally covered by platelets, whereas those from Corumbá present platelets only axially on the median region of the abdomen.

Regarding color pattern, the population from Itaipu reservoir presents greyish-blue background with light spots of variable diameter. Ventral surface of head and abdomen with whitish background and larger and darker spots of variable shapes, which are sometimes coalescent. On upper part of body spots are scattered almost homogeneously with more than one spot on each plate. All fins with rows of white spots along rays and inter-radial membranes. In live specimens these spots have orange-yellow tint. On the other hand, the population from Corumbá reservoir has brown or greyish-brown background with light spots of variable diameter. Ventral surface of head and abdomen with whitish background without spots, except ventral surface of caudal peduncle. Dots on head regularly spaced and well defined, round and sometimes fused or vermiculated. Posterior portion of body sides generally with one dot per plate, which are frequently aligned, forming well-defined horizontal lines. Unpaired fin membranes usually with variable transverse rows of white dots. In live specimens these spots have yellowish tint.

Electrophoretic analysis

Fourteen enzymatic systems were analysed, allowing detection of 38 alleles in 25 loci. Table 2 shows the enzymatic systems analysed in this work. The tissue-specific expression patterns were similar to those found in three *Hypostomus* species from the Iguaçu River by Zawadzki *et al.* (2001).

Table 3 shows the allelic frequencies of the two populations analysed. The Itaipu population is polymorphic at loci sAat-A, sAat-B, Acp-A, Gcdh-A, Gpi-A, Gpi-B, sIcdh-a, sMdh-A, and Pgm-A and the Corumbá population is polymorphic at sAat-A, sAat-B, Acp-A, sIcdh-A, sMdh-A and Pgm-A. Significant allelic frequency differences were found only in locus sAat-A ($\chi^2 = 15.99$; $P = 0.00034$). No diagnostic locus was found for these two populations. Unbiased genetic identity of Nei (1978) for the two populations was $I = 0.988$ and genetic distance was $D = 0.012$. Genetic variability for the two populations is presented in Table 4. All polymorphs loci analysed were in Hardy-Weinberg equilibrium.

Discussion

The morphological population data from the Itaipu and Corumbá reservoirs are not substantially different from those taken from the *Hypostomus margaritifer* holotype or from the description by Regan (1907). However, in the light of our results, some differences should be commented on, as well as some further studies carried out on this species.

In the original description, Regan (1907) pointed out a body background color with dispersed light spots, a relatively small number of teeth (18 to 22) and a "naked abdomen, except for some small scattered granules". This description was based only on the holotype, which is an immature form of this species, with a total length of 172.3 mm. Moreover, Ihering (1911), in his redescription of *H. margaritifer* based on adult specimens, mentioned dorsal fin size and the larger areas of scutelets in the abdomen as ontogenic differences. Nevertheless, he described a distinct subspecies, *Hypostomus margaritifer butantanis*, from a single specimen from the Tiete River basin, based only on a different color pattern. At this point, he did not imagine a possible intraspecific color pattern variation in two allopatrid populations.

Table 3. Allelic frequencies estimated for two populations of *Hypostomus margaritifer* (Regan, 1907) from the Itaipu and Corumbá reservoirs

Locus	Allele	Itaipu population (n = 15)	Corumbá population (n = 15)
sAta-A	*a*	0.367	0.867
	b	0.600	0.133
	c	0.033	----
sAta-B	*a*	0.333	0.300
	b	0.667	0.700
Acp-A	*a*	0.767	0.967
	b	0.233	0.033
Adh-A	*a*	----	0.033
	b	1.000	0.967
Gcdh-A	*a*	0.967	1.000
	b	0.033	----
G3pdh-A	*a*	1.000	1.000
G3pdh-B	*a*	1.000	1.000
G6pdh-A	*a*	1.000	1.000
G6pdh-B	*a*	1.000	1.000
Gpi-B	*a*	0.100	----
	b	0.800	1.000
	c	0.100	----
Gpi-A	*a*	0.967	1.000
	b	0.033	----
mIcdh-A	*a*	1.000	1.000
sIcdh-A	*a*	0.067	0.067
	b	0.933	0.933
Ldh-B	*a*	1.000	1.000
Ldh-A	*a*	1.000	1.000
sMdh-A	*a*	----	0.133
	b	0.867	0.767
	c	0.133	0.100
mMdh-A	*a*	1.000	1.000
sMdh-B	*a*	1.000	1.000
sMdhp-A	*a*	1.000	1.000
mMdhp-B	*a*	1.000	1.000
Per-1	*a*	1.000	1.000
Per-2	*b*	1.000	1.000
Per-3	*c*	1.000	1.000
Pgm-A	*a*	0.267	0.100
	b	0.733	0.900
Sod-A	*a*	1.000	1.000

Table 4. Genetic variability measures for 25 loci of two populations of *Hypostomus margaritifer* (Regan, 1907). Numbers in parentheses are standard deviations. N = number of analyzed specimens; K = number of alleles per locus; $P_{0.99}$ = frequency of polymorphic loci; H_o = observed heterozygosity; H_e = expected heterozygosity

Population	N	K	$P_{0.99}$	H_o	H_e
Itaipu	15	1.4 (0.1)	36.0	0.060 (0.021)	0.104 (0.035)
Corumbá	15	1.3 (0.1)	28.0	0.056 (0.024)	0.061 (0.025)

Gosline (1947) analysed two forms, referred to as *H. margaritifer* from the Mogi-Guaçu River, called *a* and *b*. On the one hand, he described form *a* as presenting dark coloration with dispersed clear spots without tendency to fusion, with a single spot on each dermal plate and the fins following the body pattern. On the other hand, form *b* had a dark background coloration, interrupted by vermiculated bright orange lines on the head, body and fins.

In the present work, color pattern differences among the populations analysed were more conspicuous than morphometric ones. Some still not understood factors seem to determine that certain species or lineages of species of the genus *Hypostomus* are conservative, whereas other species may be more variable as regards color attributes such as number, shape, size and disposition of blotches or spots on the body and the tonality of the background coloration. The intraspecific pattern variation is sometimes greater than the differences between close species; therefore, the examination of few specimens from various populations may infer an appearance of discontinuity in a determined character, when in truth the analysis of a larger sample would show a continual variation in the character.

In the upper Paraná River basin, actually known as all the stretch upstream Itaipu dam, *Hypostomus regani* (Ihering, 1905) and *Hypostomus* aff. *auroguttatus* Natterer and Heckel in Kner, 1854 share some characters with *H. margaritifer*: high dorsal fin, elongated crown of teeth and colour pattern with clear spots or vermiculations on a darker ground. According to Gosline (1947), these two species can be distinguished from *H. margaritifer* by the possession of a greater number of teeth (in the mandible: 35 to 91 and 48 to 84 versus 18 to 32, respectively) and by the rows of teeth which do not form an acute angle at the median union of the mandibles. Two other species from other parts of Paraná River system have some characters similar to *H. margaritifer* such as strong, elongated, and relatively few teeth, a color pattern with clear spots and mandibles forming an acute angle. Weber (1987) cites *H. microstomus* Weber, 1987 from the middle Rio Paraná, downstream from the former natural geographic barrier Sete Quedas, as a species probably related to *H. margaritifer*. Reis *et al.* (1990) cite *H. roseopunctatus* Reis, Weber and Malabarba, 1990 from the Uruguay River as having such common characters with *H. microstomus* but the possession of only one plate bordering the supraoccipital in *H. margaritifer* and *H. microstomus* excludes *H. roseopunctatus*, which has three plates, from a close relationship. Finally, *H. margaritifer* can be fully distinguished from *H. microstomus* by the proportion of mandibular length in the orbital diameter (1.8 to 2.8 versus equal to or greater than 4.0, respectively).

In the neotropic fish family Loricariidae recent works (Zawadzki *et al*., 1999; 2000; Zawadzki, 2001) are devoted to associate isozyme and allozyme electrophoresis with traditional morphological methods. Analyses of 12 loricariid species from the Corumbá Reservoir region (Zawadzki and Renesto, in press) showed *H. regani* as the species closest to *H. margaritifer* with a genetic identity of Nei (1978) value of 0.941. When the *H. margaritifer* population from the Itaipu reservoir was compared to 15 other Hypostominae fishes (Zawadzki, 2001), the genetic identity for a sympatric population of *H. regani* was 0.890. In both analyses, the locus Per-1 was diagnostic for a slower anodal allele migration in *H. margaritifer*.

In this study, the genetic identity (0.988) found between the two populations and the lack of any fixed allelic difference between them are indicative that these populations belong to the same species. Differences in the allelic frequencies of loci sAat-A and Gpi-B and greater mean heterozygosity of the population from Itaipu could be explained by any differential environmental biochemical response or by genetic drift caused by geographical isolation. Established differences in the allelic frequencies between the two populations led them to be considered, for biodiversity studies or conservation purposes, as distinct evolutionary units. Despite genetically analysing only two populations of the whole range of *H. margaritifer*, we suppose that the population from the Corumbá Reservoir is the most divergent. It can be attributed to the relative endemism of the Corumbá River fish fauna that was maintained by a series of rapids and canyons acting as barriers below the mouth of the Corumbá River into the Paranaíba River (Pavanelli and Britski, 1999).

Differently from the Corumbá population, the expressive greater value of expected mean heterozygosity of the Itaipu population when compared to the obtained one, could be explained by a non random behavioural mating found on small groups of organisms within the whole Itaipu population. According to Selander (1970) we have a lack of heterozygote phenotypes when populations are subdivided in small reproducing confamiliar groups.

The intraspecific complexity and variability of characters observed in most species of *Hypostomus*, and particularly in *H. margaritifer*, shows the difficulties of establishing relationships between species with several common characters. According to Zawadzki (2001), the relationships of *H. margaritifer* could be resolved only by larger genetic and morphological studies.

From a taxonomic point of view, the numerous nominal species of *Hypostomus* described from the upper Paraná River have to be reconsidered in the light of these results. It means that analysing tissue samples for molecular systematic purposes, allied to morphological studies, is essential. Without molecular systematic resources, the problem of the validity of numerous species may stand for a long time.

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