Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v45i1.66339

Enzymatic distance in two species of the genus *Pimelodella* (Siluriformes, Heptateridae) from the Upper Paraná River basin, Paraná State, Brazil

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ABSTRACT. Species of the genus *Pimelodella* (Eigenmann & Eigenmann, 1988) present many similar morphological characteristics, which makes taxonomic identification difficult. Some species present morphological distinction and reproduce normally, while others present reproductive isolation without morphological distinction. Taking that into consideration, we used starch-gel electrophoresis of enzymes to compare two species of the genus *Pimelodella* collected in Paraná, Ivaí and Pirapó rivers of the Upper Paraná River basin. We analyzed the liver and muscle tissue of 82 *Pimelodella avanhandavae* and 29 *Pimelodella gracilis* individuals, which revealed few genetic differences between species. In addition, we carried out an experimental design based on the separation of four populations: *P. avanhandavae*, Parana River (n = 37); *P. avanhandavae*, Ivaí River (n = 6); *P. gracilis*, Ivaí River (n = 29); *P. avanhandavae*, Pirapó River (n = 39). The high proportions of polymorphic *loci* as well as the expected heterozygosity suggest that both species have high genetic variability. Nei's genetic identity values and Wright's fixation indices revealed a large genetic proximity between the samples collected in Ivaí and Pirapó rivers, regardless of the species analyzed. The presence of fixed alleles at one hundred percent for two *loci* showed that *P. avanhandavae* individuals collected in the Parana River are genetically distant from all remaining populations. Data indicated that *P. avanhandavae*, collected in this river, constitutes a distinguished species from those collected in Ivaí and Pirapó rivers. Therefore, we suggest, that more studies with molecular markers be carried out with the genus, as well as a review of the diagnostic morphological characteristics used to separate *P. avanhandavae* from *P. gracilis*.

Keywords: diagnostic features; genetic distance; genetic variability; Paraná River basin; *Pimelodella avanhandavae*; *Pimelodella gracilis*.

Received on December 18, 2022.
Accepted on September 13, 2023.

Introduction

Different specimens of the *Pimelodella* genus have a few of morphological similarities, which impedes the process of species identification. According to Langeani et al. (2007), the native *Pimelodella* species presenting "type locality" in Upper Paraná are *Pimelodella avanhandavae* (Eigenmann, 1917), *Pimelodella boschmai* (Van der Stigchel, 1964), *Pimelodella meeki* (Eigenmann, 1910), and *Pimelodella rudolphi* (Miranda-Ribeiro, 1918). In addition, *Pimelodella gracilis* (Valenciennes, 1835) is also considered native, but with a different "type locality" from Upper Paraná. *Pimelodella taenioptera* (Miranda-Ribeiro, 1914), in turn, was probably dispersed with the construction of Itaipu dam to other basins in the Neotropics (Langeani et al., 2007; Tonella et al., 2022).

Science used the morphological differences of the species as basic parameters to identify a species throughout the 19th and early 20th centuries (Keat-Chuan Ng, Aun-Chuan Ooi,Wong, & Khoo, 2017). Species are the main unit of evolution and some development issues cannot be approached without clarifying their meaning. Mayr (1963) considers that species are organisms of sexual reproduction, "groups of natural populations, real or potentially interbreeding, reproductively isolated from other groups", but which constitute a 'green drive' and yet 'genetic unit', formed by its extensive heritage gene in intercommunication. This reproductive isolation is used as an adaptation to protect the well-integrated genes and coadapted species.

Even though differences in animal morphology derive from genetic variation, the environment may also influence the characteristics of an individual. Furthermore, there are many populations that differ morphologically without presenting reproductive isolation, which are the sibling species (Singh, 2016) or isolated in reproductive conditions, without extensive morphological differentiation, which are the cryptic species (Artaev et al., 2021; Petrosino et al., 2022). Within the genus *Pimelodella* there is a conserved morphology and ride distribution, representing one of the most difficult bottlenecks for understanding current Neotropical river. It results in a great difficulty to identify several individuals found in scientific collections. Even identification in location already sampled is a challenge (Slobodian, Akama, & Dutra, 2017).

Many are the difficulties remaining to identify the genus *Pimelodella* by means of morphological characters once it possesses the largest number of species of the Heptapteridae family, around 82 valid ones (Terra et al., 2022). There are also the voucher species that were not filed yet (Dazzani, Garcia, Peixoto, Trajano, & Almeida-Toledo, 2012). The traditional classification based on morphology needs to be revised (Slobodian et al., 2017; Conde-Saldaña, Albornoz-Galzón, García-Melo, & Villa-Navarro, 2019; Alves, Chambrier, Luque, & Scholz, 2020). In this context, our study established a comparison between two species of the genus *Pimelodella* (*P. avanhandavae* and *P. gracilis*) found in Upper Paraná River basin via genetic variability of 12 enzyme systems.

Material and Methods

Specimens were collected under permits from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), number 37181-1/2013. Captured individuals were frozen in liquid nitrogen in accordance with the Ethical Principles in Animal Research guidelines adopted by the National Council of Control of Animal Experimentation (CONCEA). We collected 82 specimens of *P. avanhandavae* and 29 of *P. gracilis* at the Upper Paraná River basin, from March to October 2012 (Figure 1). Species identification followed Ota, Deprá, Graça, and Pavanelli (2018).

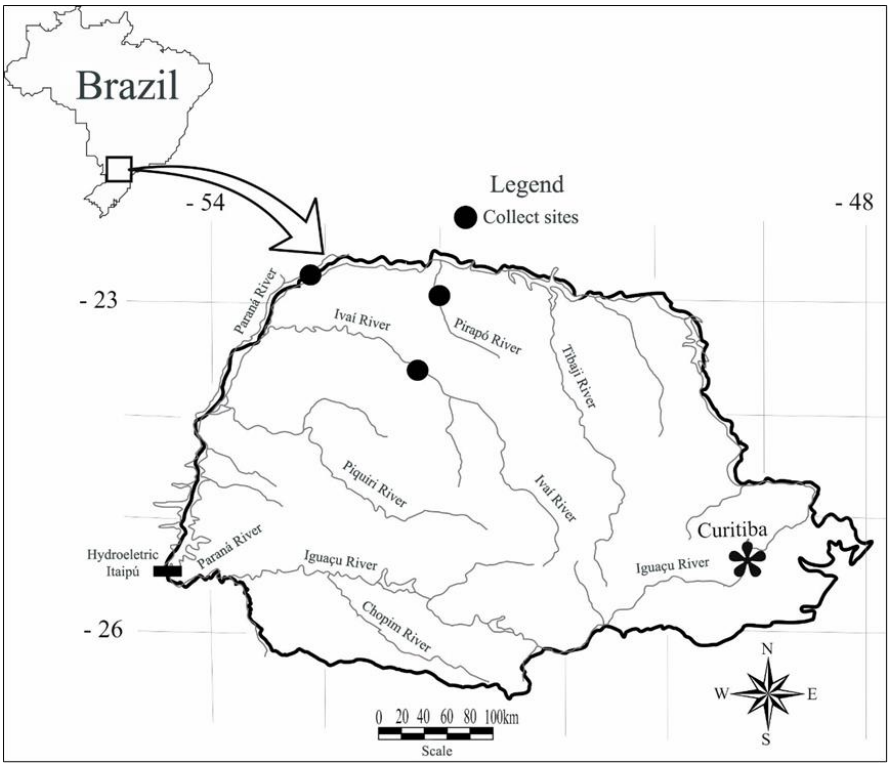

Figure 1. Map of the State of Paraná indicating the three collection sites: Paraná River, Pirapó River, and Ivaí River, indicated by a black circle, as per legend.

The diagnostic characteristic distinguishing *P. avanhandavae* from *P. gracilis* is the presence of a dorsal strip in *P. avanhandavae* (XXIX plate in Eigenmann, 1917), which extends over virtually the entire length of the back body, from the end of the head to the end of adipose fin on both sides of the dorsal fin and fat (Ota et al., 2018), as shown in Figure 2.

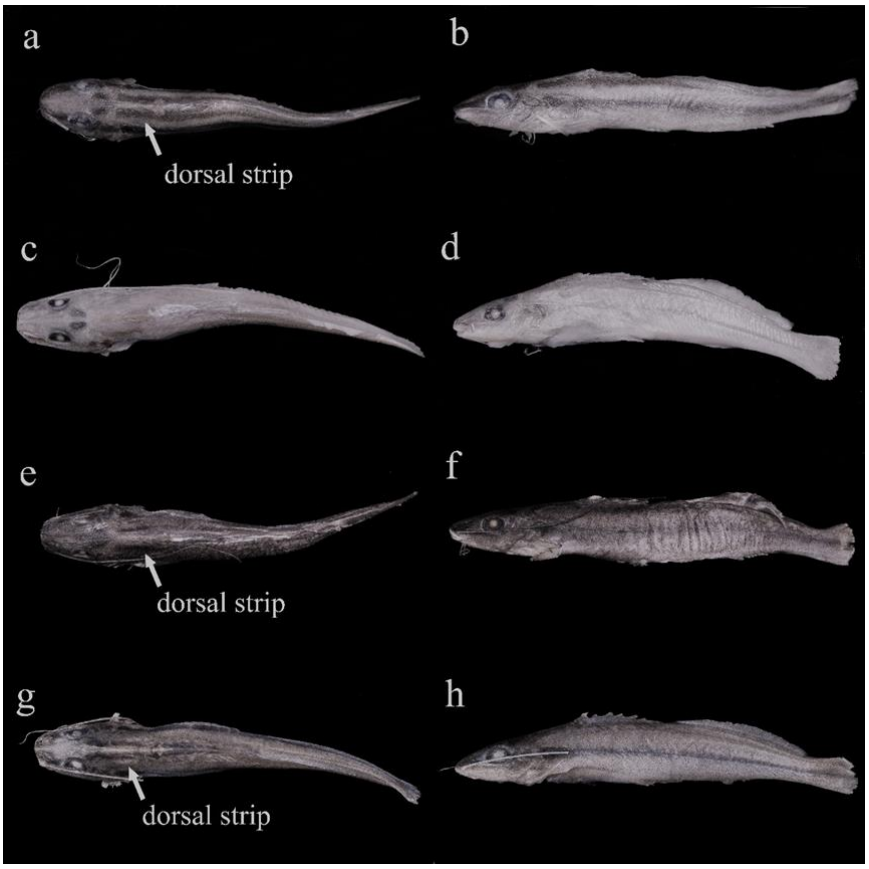

Figure 2. *Pimelodella avanhandavae* from Paraná River (a, b); *Pimelodella. gracilis* from Ivaí River (c, d); *Pimelodella avanhandavae* from Ivaí River (e, f); and *Pimelodella avanhandavae* from Pirapó River (g, h). Presence of dorsal strip in *Pimelodella avanhandavae* as diagnostic characteristics.

To reach a full differentiation analysis, we compared not only different species, but also populations of the same species from different locations. Thus, four populations were analyzed: *P. avanhandavae*, from Parana River (n = 37); Ivaí River (n = 6), and Pirapó River (n = 39) as well as *P. gracilis* from Ivaí River (n = 29) (Table 1).

Table 1. General characteristics of the sampling sites and the four populations, indicating the place, municipality, geographic coordinates, and number of samples for populations 1, 2, 3, and 4.

Populations	Collection site	City	Geographic coordinates	Sample size
Population 1 *P. avanhandavae*	Paraná River	Porto Rico (PR)	22°45'51.80" S 53°15'25.20" W	37
Population 2 *P. avanhandavae*	Ivaí River	Ivatuba (PR)	23°38'05.05" S 52°14'48.40" W	6
Population 3 *P. gracilis*	Ivaí River	Ivatuba (PR)	23°38'05.05" S 52°14'48.40" W	29
Population 4 *P. avanhandavae*	Pirapó River	Jardim Olinda (PR)	22°32'54.66" S 52°01'41.99" W	39

After the collection, the individuals were quickly immersed in liquid nitrogen (-196°C) and stored in a cryogenic container. The specimens were properly transported and stored at low temperature (-20°C) to have the liver and muscle tissues enzymes extracted according to methodology adapted from Paiva, Zawadzki, Ruvolo-Takasusuki, Lapenta, and Renesto (2013). Liver and muscle tissue of all animals were removed and macerated in separate plastic 1.5 mL microtubes containing 100 µL of Tris/HCl 0.02 M pH 7.5. Because of large amounts of fat in the liver, ratio 1: 2 (fabric: CCl4) was added with carbon tetrachloride (CCl4) for the precipitation to occur.

Once immersed, the tubes were subjected to a twenty-minute centrifugation at 12.000 RPM (16.128g) at a temperature between 1°C and 5°C. The protein extract (supernatant) was applied to starch gel with Whatman 3M paper filter with strips of 7 x 3 mm. Starch gel preparation used cornstarch® Penetrose 50 to 15% diluted 15 times in buffer gel – the use of buffers depended on the enzymes revealed. To prepare the gel, a dilute

Penetrose buffer solution was heated to the boil point and was poured on a glass plate with an edge of 0.6 cm. After reaching room temperature, the gels remained under cooling for about 24 hours followed by continuous horizontal electrophoresis under a sixteen-hour refrigeration systems Tris-citrate 0.135 - 0.043M buffer pH 7.0 (TC) and Tris-borate-EDTA pH 8.6 0.18-0.10004M (TBE).

The voucher specimens were stored at the collection of Nupelia (*Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura* – Center of Research in Limnology, Ichthyology and Aquaculture) of the State University of Maringá under numerical identification: NUP14527 for population of *P. avanhandavae* from Paraná River; NUP14528 for population of *P. avanhandavae* from Ivaí River; NUP14529 for population of *P. gracilis* from Ivaí River, and NUP14530 for population of *P. avanhandavae* from Pirapó River.

Loci and alleles were named according to Simonsen (2012) and data analyzed using Popgen 1:32 software (Yeh, Yang, Boyle, Ye, & Mao, 1997). Genetic variability was determined by calculating heterozygosity (expected and observed) according to Nei (1978). With the values of the allele frequencies, the identity (I) and the genetic distance (D) were calculated and was carried out by means of dendrogram (grouping method by the algorithm UPGMA- Unweighted Pair Group Method with Arithmetic Means) of the populations, assuming Hardy-Weinberg equilibrium.

Results and discussion

Polymorphism values and Isoenzymes patterns

Among the 12 enzyme systems (AAT, ACP, ADH, G3PD, GDH, GPI, HDI, LDH, MDH, PER, PGM, SOD) analyzed in 111 individuals of the genus *Pimelodella*, we identified 21 *loci* and 41 alleles. *Aty-2, Acp-G3pd-3, Gdh, Gpi-1, Gpi-2, Idh-2, Mdh-2, Per-1, Per-2, Pgm-2*, and *Sod*) with a percentage of 57.14% of polymorphic *loci. Pimelodella* polymorphism proved higher than the rates found in other studies. Limeira, Renesto, and Zawadzki (2009) estimated the values of 13.64 and 9.09% of polymorphism for *Rineloricaria pentamaculata* from two sites of the Paraná River basin. In contrast, it was lower than the values found by Lidani, Torres, Madeira, Carneiro, and Gabriel (2018) for *Astyanax sp.* from Iguaçu River (95,2%) as well as below the rate found by Lucena, Renesto, Oliveira, Mateus, and Zawadzki (2012), who detected a percentage of 50% of polymorphic *loci* for *Neoplecostomus* from nine sites.

The enzymes analyzed had different electrophoretic patterns as follows: AAT with two *loci*, a polymorphic and a monomorphic one, and activity in both muscle and liver. ACP with only one band per individual (by comparing the position of the bands the presence of three distinct alleles is revealed, suggesting a single polymorphic locus). ADH with alcohol dehydrogenase, also a dimeric enzyme with activity only in the liver, but encoded by a single monomorphic locus expressed by means of a cathodic band.

G3PD, in the enzyme Glycerol-3-phosphate dehydrogenase, indicated three *loci*, two polymorphic and one monomorphic, with higher activity in the liver tissue in relation to the muscle tissue, indicating no genetic variability. The Paraná River population was the only to present the allele *G3pd-3* (*b*), while in Ivaí and Pirapó Rivers only the *G3pd-3* (*a*) allele was observed. GDH is a monomeric enzyme which presented a single band at the three sites, except for a sample from Paraná River with two bands.

GPI had a significant expression in both the muscle and the liver; it is a dimeric enzyme codified by two polymorphic *loci*. The most anodic locus is best expressed in the liver tissue, while the least anodic one is best expressed in the muscle tissue. Along with the enzyme IDH, GPI presented the highest polymorphism of all enzymes analyzed. IDH is a dimeric enzyme expressed through a two-locus pattern: the most anodic in the liver tissue and least anodic in the muscle tissue. Among the polymorphic *loci* in our studies, three had higher polymorphism (*Gpi-1, Gpi-2*, and *Idh-2*). For our study, the authors considers that the enzymatic polymorphism may be attributed to the variety of physiological interactions.

Our results allow to conclude that *P. avanhandavae* and *P. gracilis* have high genetic variability (Table 2), when compared to other research (Limeira et al., 2009), and the three locations presented He values above the average (0.051) for freshwater fish (Ward, Skibinski, & Woodwark, 1992). The frequencies of polymorphic *loci* (Table 2) were also regarded as high (from 28.57 to 38.10%). Hardy-Weinberg's balance was verified in eight (34.78%) of the *loci* where some type of allele polymorphism had been detected: for Paraná River, only *Per-1*; Ivaí River *P. avanhandavae* population, only *Gpi1, Pgm-2*, and *Idh-2*; for Pirapó River population, *Gpi-1, Aat-2*, and *Mdh-2*, and for *P. gracilis*, only *Gpi-1*.

Table 2. Allele frequencies at 21 *loci* of three populations of *Pimelodella avanhandavae* and one population of *Pimelodella gracilis*. P = proportion of polymorphic *loci*; K = mean number of alleles ± Standard deviation.

Loci	Alleles	*P. avanhandavae*			*P. gracilis* (n=29)
		Paraná River (n=37)	Ivaí River (n=6)	Pirapó River (n=39)	
Aat-1	*A*	1.0000	1.0000	1.0000	1.0000
Aat-2	*A*	0.0270*			
	B	0.9054	0.1667*	0.0256	
	C	0.0676	0.8333	0.9744	1.0000
Acp	*A*	0.0676*	0.3333*	0.2308*	0.4828*
	B	0.5405	0.5000	0.6923	0.4828
	C	0.3919	0.1667	0.0769	0.0345
Adh	*A*	1.0000	1,0000	1.0000	1.0000
G3pd-1	*A*	1.0000	1.0000	1.0000	1.0000
G3pd-2	*A*	1.0000	1.0000	1.0000	1.0000
G3pd-3	*A*		1.0000	1.0000	1.0000
	B	1.0000			
Gdh	*A*	0.9730*	1.0000	1.0000	1.0000
	B	0.0270			
Gpi-1	*A*	0.0135*	0.0833	0.0641	0.0517
	B	0.8784	0.9167	0.8846	0.9483
	C	0.1081		0.0513	
Gpi-2	*A*		0.1667*	0.3205*	0.4259*
	B	0.4167*	0.8333	0.4487	0.5741
	C	0.5833		0.2308	
Idh-1	*A*	1.0000	1.0000	1.0000	1.0000
Idh-2	*A*	0.2167*	0.1000	0.0676*	0.0192*
	B	0.3667		0.0541	0.0577
	C	0.3500	0.3000	0.3919	0.2500
	D	0.0667	0.6000	0.4865	0.6731
Ldh-1	*A*	1.0000	1.0000	1.0000	1.0000
Ldh-2	*A*	1.0000	1.0000	1.0000	1.0000
Mdh-1	*A*	1.0000	1.0000	1.0000	1.0000
Mdh-2	*A*			0.0385	
	B	1.0000	1.0000	0.9487	1.0000
	C			0.0128	
Per-1	*A*	0.0946	1.0000	1.0000	1.0000
	B	0.9054			
Per-2	*A*	0.0811*	0.3333*	0.0256*	0.1724*
	B	0.8919	0.6667	0.9744	0.8276
	C	0.0270			
Pgm-1	*A*	1.0000	1.0000	1.0000	1.0000
Pgm-2	*A*	1.0000	1.0000	1.0000	0.9655*
	B				0.0172
	C				0.0172
Sod	*A*		1.0000	1.0000	1.0000
	B	1.0000			
P		38.10	28.57	33.33	28.57
K		1.6667 ± 0.9661	1.3810 ± 0.6690	1.6190 ± 0.9735	1.4286 ± 0.8106

* Statistically significant values.

As well as GPI, it contributed to the high heterozygosity average expected for the three sites. Only one band appeared in the muscle tissue, which did not occur for the liver tissue, in which its expression was stronger, enabling the detection of two *loci*, a polymorphic (*Idh 2*) and monomorphic (*Idh 1*) one. When considered the metabolic role of these enzymes, polymorphism may have a regulatory function, since the heterozygous organism can modulate its reaction to variable conditions, which suggests that the enzymatic polymorphism has selective role.

Other authors had also found high polymorphism in GPI (Lucena et al., 2012). IDH is one of the catalyst enzymes in Kreb's cycle (Munin, Aquino-Silva, Schwantes, Almeida-Val, & Sato 2012), therefore involved in the entire cell metabolism, which explains its good expression both in the liver and the muscle, as well as GPI, which takes part in important metabolic processes. A good polymorphism in the *loci* which express these enzymes may lead the individuals to a great adaptive success in environmental stress conditions (Griffiths, Doebley, Peichel, & Wassarman, 2022).

LDH is a tetrameric enzyme with a pattern of *interloci* heterotetrametric bands in some individuals. The analysis of both liver and muscle tissues suggests the expression of two *loci* in homozygosis. MDH is a dimeric enzyme with a three-band pattern, typical to an enzyme codified by two *loci*. We verified polymorphism only for the most anodic locus found in the liver with the detection of the second locus transcribing this enzyme. It presented homozygosis in the populations from Paraná and Pirapó Rivers.

PER has an electrophoretic pattern which implies a codification by two polymorphic *loci*. Only the population of Paraná River proved polymorphic for the locus *Per-1*. PGM is an enzyme with two *loci*, one monomorphic in the populations of Paraná and Pirapó Rivers and another polymorphic one for the population of Ivaí River. SOD is an enzyme codified by a monomorphic locus, with two different alleles fixed in each population. The population of Paraná River appeared fixed for the allele *Sod-b*, while the populations of Ivaí and Pirapó Rivers are fixed for the allele *Sod-a*.

Fixation indices and genetic distance

No significant differences were identified separating the two species. The locus *Pgm-2* is highlighted for presenting a second allele in the population of *P. gracilis* at a low frequency (*Pgm-2(b)* = 0.0172). The populations also presented specificities regarding some of the *loci* identified. We observed that the enzymes G3PD and SOD had fixed alleles for the population of Paraná River and for the *loci G3pd-3* and *Sod*, in addition to some exclusive alleles at low frequency (0.0270) in the *loci Aat-2* and *Per-2,* according to the Table 3, with Wright's fixation indices (1949).

Table 3. Wright's fixation indices (1949) for populations of *Pimelodella avanhandavae* from Paraná, Ivaí, and Pirapó Rivers, Paraná State, Brazil.

Loci	Paraná River		Ivaí River		Pirapó River		Total		
	N	Fis	N	Fis	N	Fis	Fis	Fit	Fst
Aat-1	37		6		39				0.0000
Aat-2	37	0.8455*	6	1.0000*	39		0.8442*	0.9451*	0.6475*
Acp	37	0.9508*	6	1.0000*	39	1.0000*	0.9833*	0.9844*	0.0630*
Adh	37		6		39				0.0000
G3pd-1	37		6		39				0.0000
G3pd-2	37		6		39				0.0000
G3pd-3	37		6		39			1.0000*	1.0000*
Gdh	37	1.0000*	6		39		1.0000*	1.0000*	0.0182
Gpi-1	37	0.0017	6	-0.0909	39	0.1482*	0.0305	0.0458*	0.0158
Gpi-2	36	1.0000*	6	1.0000*	39	0.9601*	0.9818*	0.9852*	0.1907*
Idh-1	37		6		39				0.0000
Idh-2	30	0.4699*	5	0,6296	37	0.6410*	0.5731*	0.6248*	0.1211*
Ldh-1	37		6		39				0.0000
Ldh-2	37		6		39	-0.0263			0.0000
Mdh-1	36		6		39				0.0000
Mdh-2	37		6		39	-0.0435*	-0.0435*	-0.0141	0.0282
Per-1	37	0.2111*	6		39		0.2111*	0.8931*	0.8645*
Per-2	37	1.0000*	6	1.0000*	39	1.0000*	1.0000*	1.0000*	0.1318*
Pgm-1	37		6		39				0.0000
Pgm-2	37		6		39				0.0000
Sod	37		6		39			1.0000*	1.0000*
Mean							0.7332	0.8572	0.4649

*- statistically significant values.

The populations of Ivaí (*P. avanhandavae* and *P. gracilis*) and Pirapó Rivers revealed monomorphic *loci* which distance them from the population of Paraná River (*Gdh, Idh-1* and *Per-1*). Furthermore, even though these two populations have similar allele structuring, the three of them differ in frequency for some *loci* (*Acp, Gpi-2* and *Idh-2*). The results obtained point out that some *loci* are Hardy-Weinberg's balance: *Per-1* for Paraná River population, *Gpi-1, Pgm-2* for *P. gracilis* from Ivaí River, *Gpi-1, Idh-2* for *P. avanhandavae* from Ivaí River, and *Aat-2*, *Gpi-1* and *Mdh-2* from Pirapó River. More *loci* in heterozygosity are expected for *P. avanhandavae*, as well as higher averages, indicating greater genetic variability in *P. avanhandavae*.

The mean heterozygosity expected (He) and measured (Ho) for the populations had respective He values = 0.1227± 0.2103, Ho = 0.0368 ± 0.0929, for the *P. avanhandavae* population from Paraná River, He = 0.1202 ± 0.2177, Ho = 0.0175 ± 0.0554, for Ivaí River population, He = 0.1045 ± 0.2060, Ho = 0.0298 ± 0.0619 for Pirapó River population, and He = 0.0947 ±0.1861 and Ho= 0.0157± 0.0399 for the population of *P. gracilis*. In

addition, we observed an accumulation of homozygous in the following *loci*: *Aat-1, Adh, G3pdh-1, G3pdh-2, G3pdh-3, Idh-1, Ldh-1, Ldh-2, Mdh-1, Pgm-1,* and *Sod.* The values of Fst for the *loci Aat-2, G3pd-3, Per-1,* and *Sod* indicated differentiation among some of the *loci* of the four populations.

When confronted the mean expected heterozygosity (He) *P. avanhandavae* from Ivaí River and *P. gracilis,* we see higher values for *P. avanhandavae*. Another particularity, among very few differences found between the two species, was the presence of an exclusive allele in the locus *Pgm-2* of *P. gracilis*, allele *b*, with a frequency of 0.0172 (*Pgm-2 a*= 0.9828 and *Pgm-2 b*= 0.0172), which may indicate the presence of recent mutations. According to Ota et al. (2018), freshwater fish are very dynamic, and species differentiations often appear, as demonstrated by several authors (Alves et al., 2020; Katz & Costa, 2020; Azevedo et al., 2021).

The test for genetic balance revealed 19 *loci* as well as 26 polymorphic, which are not in Hardy-Weinberg's balance. The populations had significant differences for the *loci Aat-2, Acp, G3pd-3, Gdh, Gpi-2, Idh-2, Per-1, Per-2,* and *Sod*, according to the indices of Fst and corroborated through chi-square homogeneity tests, highlighting *loci Aat-2, G3pd-3, Per-1,* and *Sod*, which indicated Fst values above 0.25, thus demonstrating a high rate of differentiation among the populations (Wright, 1949).

Genetic identity

The values of distances and genetic identities proposed by Nei in 1978 (Table 4) indicated no significant differences between the species *P. avanhandavae* and *P. gracilis*. The individuals removed from Ivaí River and separated according to a taxonomic classification proved great genetic similarity (I = 0.9921). Despite being formed by different species, the samples from Ivaí and Pirapó Rivers presented high similarity indices as well (I = 0.9920 between Ivaí - *P. gracilis* and Pirapó - *P. avanhandavae* and I = 0.9856 between Ivaí - *P. avanhandavae* and Pirapó - *P. avanhandavae*).

Table 4. Nei's identity measure (1978) (above) and distances (below) for populations of *Pimelodella avanhandavae* and *Pimelodella gracilis* from Paraná, Ivaí, and Pirapó Rivers, Paraná State, Brazil.

Population	Paraná *P. avanhandavae*	Ivaí *P. avanhandavae*	Pirapó *P. avanhandavae*	Ivaí *P. gracilis*
Paraná River *P. avanhandavae*	*****	0.7871	0.7883	0.7682
Ivaí River *P. avanhandavae*	0.2394	*****	0.9887	0.9953
Pirapó River *P. avanhandavae*	0.2379	0.0114	*****	0.9928
Ivaí River *P. gracilis*	0.2637	0.0047	0.0072	*****

These mutations, which generate genetics polymorphisms, can cause, in favorable environment, speciation among phenotypically similar individuals, making identification by morphology difficult. (Vanhaecke et al., 2012). Ronqui, Galhardo, Lisboa, Ruvolo-Takasusuki, and Toledo (2016) states that isoenzymes can provide important information of species differentiation. Nei's genetic identity values (1978) found in this study for the populations from Ivaí and Pirapó Rivers are particular to co-specific populations. Two populations regarded as co-specific should have values above 0.85 for genetic identity (Thorpe & Solé-Cava, 1994).

Based on Nei's genetic distances (1978) we were able to design a dendrogram (Figure 3) showing the distances in centimeters among the four populations. When analyzing the genetic identity value found between the populations of *P. avanhandavae* and *P. gracilis* from Ivaí River (I = 0.9953) as well as the value found for the populations of *P. gracilis* from Ivaí River and *P. avanhandavae* from Pirapó River (I = 0.9928), we observe that they are practically identical and even higher than the value of genetic identity found between populations of the same species (Ivaí - *P. avanhandavae* and Pirapó - *P. avanhandavae* I = 0.9887).

In order for two morphotypes to be regarded as co-specific, they must have similar allele frequency per locus (Thorpe & Solé-Cava, 1994). Data indicate 100% of fixed alleles for the *loci G3pd-3* and *Sod* in the population of Paraná River, which makes it distant from the others. Among diploid organisms, the detection of fixed alleles in different populations reflects the restriction in the gene flow. Divergences between the samples from Paraná and Pirapó Rivers may be explained by the presence of hydroelectric plant of Rosana (located at Paranapanema River, between the municipalities of Rosana, São Paulo State, and Diamante do Norte, Paraná State) which has been hampering the migration of species from one river to the other.

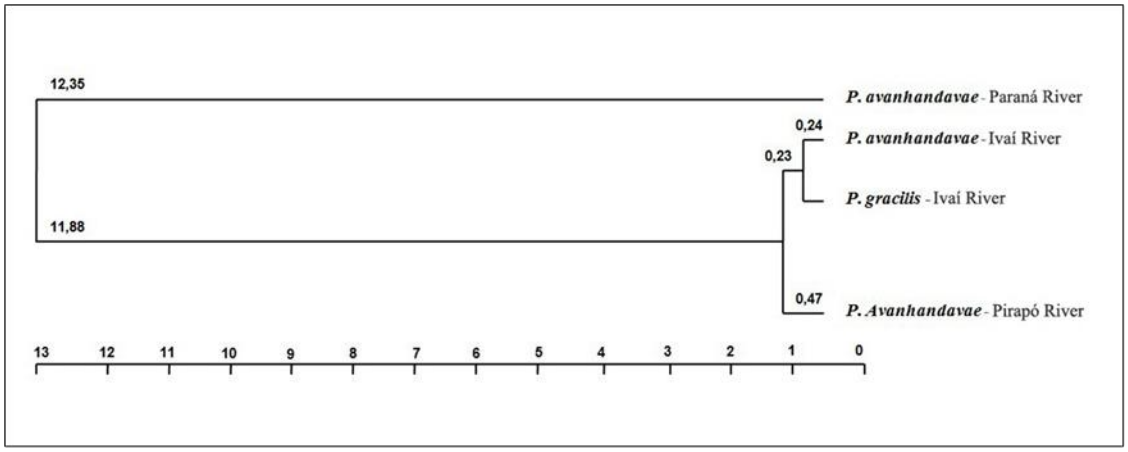

Figure 3. Dendrogram based on Nei's genetic distances (1978), UPGMA method, for the populations from Paraná (*P. avanhandavae*), Ivaí (*P. avanhandavae*), Ivaí (*P. gracilis*), and Pirapó (*P. avanhandavae*) Rivers, state of Paraná, Brazil, showing the genetic distance of the *P. avanhandavae* population from the Parana River from other populations of the same species.

This hypothesis corroborates the assumptions by Bem, Ribolli, Röpke, Winemiller, and Zaniboni-Filho (2021), who concluded that dams cause profound changes not only in taxonomic structures of fishes, but also create large-scale environmental heterogeneity that apparently determines the distribution of fish trophic guilds and food web structure. In addiction other authors also claim that dams impact river ecosystems by reducing river longitudinal connectivity (Agostinho, Gomes, Santos, Ortega, & Pelicice, 2016; Forsberg et al., 2017; Barbarossa et al., 2020).

The genetic differences between individuals of the same species from Ivaí and Paraná Rivers result from the location of the collection site in Paraná River, roughly 80 kilometers downstream the mouth of Ivaí River. Therefore, even if the fish from Ivaí River migrate to Paraná River, it is unlikely that they mix with the population sampled from the latter. Thus, the samples from our study proved genetically isolated in Paraná River from the samples from the other rivers studied, with different allele, heterozygous and polymorphic frequencies. These individuals also presented two exclusive alleles – *Aat-2 b* and *Per-2(c)* – at a low frequency (0.0270).

Conclusion

The gene flow restriction may lead to gene differentiation, which could have occurred with the population of Paraná River. Therefore, the results suggest that the *P. avanhandavae* from Paraná River is not co-specific from the populations of *P. avanhandavae* from Ivaí and Pirapó Rivers. As well that the diagnostic morphological characteristics used to separate *P. avanhandavae* from *P. gracilis* require a proper review.

Acknowledgments

The authors would like to thank *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) Brasília, Distrito Federal, Brazil, *Fundação Araucária* (Paraná State, Brazil) and the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) (Protocol No. 351071/2022-9) for the financial support. They also are thankful to Weferson J. da Graça for helping in sample collecting.

References

Agostinho, A. A., Gomes, L. C., Santos, N. C., Ortega, J. C., & Pelicice, F. M. (2016). Fish assemblages in Neotropical reservoirs colonization patterns impacts and management. *Fisheries Research*, *173*(1), 26-36. DOI: https://doi.org/10.1016/j.fishres.2015.04.006

Artaev, O. N., Ermakov, O. A., Vekhov, D. A., Konovalov, A. F., Levina, M. A., Pozdeev, I. V., ... Levin, B. A. (2021). Genetic screening of distribution pattern of *Roaches rutilus* and *R. lacustris* (Cyprinidae) in broad range of secondary contact (Volga Basin). *Inland Water Biology*, *14*, 205-214. DOI: https://doi.org/10.1134/S1995082921020024

Alves, P. V., Chambrier, A., Luque, J. L., & Scholz, T. (2020). Integrative taxonomy reveals hidden cestode diversity in *Pimelodus* catfishes in the Neotropics. *Zoologica Scripta*, *50*(2), 210-224. DOI: https://doi.org/10.1111/zsc.12465

Azevedo, F. M., Zawadzk, C. H., Soria, T. V., Fabrin, T. M. C., Oliveira, A. V., Pinto, S. M. A. & Prioli, A. J. (2021). Integrative taxonomy reveals the historically poorly-defined armored catfish *Hypostomus variipictus* (Ihering 1911), from the upper rio Paraná basin, Brazil (Siluriformes, Loricariidae). *Journal of Fish Biology*, *99*(1), 143-152. DOI: https://doi.org/10.1111/jfb.14706

Barbarossa, V., Schmitt, R. J., Huijbregts, M. A., Zarfl, C., King, H., & Schipper, A. M. (2020). Impacts of current and future large dams on the geographic range connectivity of freshwater fish worldwide. *Proceedings of the National Academy of Sciences*, *117*(7), 3648-655. DOI: https://doi.org/10.1073/pnas.1912776117

Bem, J., Ribolli, J., Röpke, C., Winemiller, K. O., & Zaniboni-Filho E. (2021). A cascade of dams affects fish special distributions and functional groups of local assemblages in a subtropical river. *Neotropical Ichthyology*, *19*(3), 1-18. DOI: https://doi.org/10.1590/1982-0224-2020-0133

Conde-Saldaña, C. C., Albornoz-Galzón, J. G., García-Melo, J. A. D., & Villa-Navarro, F. A. (2019). A new species of *Pimelodella* Eigenmann & Engenmann, 1888 (Siluriformes: Heptapteridade) from de Sierra Nevada de Santa Marta, Colombia. *Zootaxa*, *4668*(4), 562-574. DOI: https://doi.org/10.11646/zootaxa.4668.4.8

Dazzani, B., Garcia, C., Peixoto, M., Trajano, E., & Almeida-Toledo, L. F. (2012). Cytogenetic and molecular analyses in troglobitic and epigean species of *Pimelodella* (Siluriform: Heptapteridae) from Brazil. *Neotropical Ichthyology*, *10*(3), 623-632. DOI: https://doi.org/10.1590/S1679-62252012005000020

Forsberg, B. R., Melack, J. M., Dunne, T., Barthem, R. B., Goulding, M, Paiva, R. C. D., ... Weisser, S. (2017). The potential impact of new Andean dams on Amazon fluvial ecosystems. *PLoS ONE*, *12*(8), 1-35. DOI: https://doi.org/10.1371/journal.pone.0182254

Griffiths, A. J. F., Doebley, J., Peichel, C., & Wassarman, D. A. (2022). *Introdução à genética* (12. ed.). Rio de Janeiro, RJ: Guanabara Kogan.

Katz, A. M., & Costa, W. J. E. M. (2020). A new species of the catfish genus *Cambeva* from the Paranapanema river drainage, southeastern Brazil (Siluriformes: Trichomycteridae). *Tropical Zoology*, *33*(1), 2-13. DOI: https://doi.org/10.4081/tz.2020.63

Langeani, F., Correa e Castro, M. R., Oyakawa, O. T., Shibatta, O. A., Pavanelli, C. S., & Casatti, L. (2007). Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras. *Biota Neotropica*, *7*(3), 181-197. DOI: https://doi.org/10.1590/S1676-06032007000300020

Lidani, K. C. F., Torres, R. A., Madeira, F. H. M., Carneiro, P. C. F., & Gabriel, J. E. (2018). Identificação de polimorfismos genéticos em populações nativas de lambaris *Astyanax* sp. B do Rio Iguaçu (Estado do Paraná, Brasil) por análise de amplificação aleatória. *Visão Acadêmica*, *19*(3), 28-37. DOI: https://doi.org/10.5380/acd.v19i3.61124

Limeira, D. M., Renesto, E. & Zawadzki, C. H. (2009). Allozyme comparison of two populations of *Rineloricaria* (Siluriformes, Loricariidae) from the Ivaí River, upper Paraná River basin, Brazil. *Genetic and Molecular Biology*, *32*(2), 431-435. DOI: https://doi.org/10.1590/S1415-47572009005000022

Lucena, A. L. M., Renesto, E., Oliveira, C., Mateus, R. P., & Zawadzki, C. H. (2012). Genetic differentiation among ten populations of the genus *Neoplecostomus* (Teleostei: Loricariidae) from the upper Paraná River basin. *Biochemical Systematics and Ecology*, *44*, 325-332. DOI: https://doi.org/10.1016/j.bse.2012.06.020

Mayr, E. (1963). *Animal especies and evolution*. Cambridge, UK: Harvard University Press.

Munin, F. S., Aquino-Silva, M. R., Schwantes, L. B., Almeida-Val, V. M. F., & Sato, Y. (2012). Temporal Expression of Izoenzymes, Alozymes and Metabolic Markers at the Early Ontogeny of *Prochilodus argenteus* (Characidae- Prochilodontidae) from São Francisco Basin, Três Marias, Minas Gerais, Brazil. In K. Ghowsi (Ed.), *Eletrophoresis* (p. 221-234). London, UK: InTech.

Simonsen, V. (2012). Isozymes: Application for population genetics. In K. Ghowsi (Ed.), *Eletroforesis* (p. 97-114). London, UK: InTech.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89*(3), 583-590. DOI: https://doi.org/10.1093/genetics/89.3.583

Keat-Chuan Ng, C., Aun-Chuan Ooi, P., Wong, W. L., & Khoo, G. (2017). A review of fish taxonomy conventions and species identification techniques. *Survey in Fisheries Sciences*, *4*(1), 54-93. DOI: https://doi.org/10.18331/SFS2017.4.1.6

Ota, R. R., Deprá, G. C., Graça, W. J., & Pavanelli, C. S. (2018). Peixes da planície de inundação do alto rio Paraná e áreas adjacentes: Revised, annotated and updated. *Neotropical Ichthyology*, *16*(2), 1-111. DOI: https://doi.org/10.1590/1982-0224-20170094

Paiva, S., Zawadzki, C. H., Ruvolo-Takasusuki, M. C. C., Lapenta, A. S., & Renesto, E. (2013). Allozyme analysis of the four species of *Hypostomus* (Telleostei: Loricariidae) from the Ivaí river, upper Paraná rover basin, Brazil. *Acta Scientiarium. Biological Sciences*, *35*(4), 571-578. DOI: https://doi.org/10.4025/actascibiolsci. v35i4.16355

Petrosino, G. Tancioni, L., Turani, M., Rakaj, A., Ciuffardi, L., & Rossi, A. R. (2022). Phylogeopgraphy of *Sarmarutilus rubilio* (Cypriniformes: Leuciscidae): Complex genetic structure, clue to a new cryptic species and fuerther insights into roaches phylogeny. *Genes*, *13*(2), 1-23. DOI: https://doi.org/10.3390/genes13061071

Ronqui, L., Galhardo, D., Lisboa, F. T., Ruvolo-Takasusuki, M. C. C., & Toledo, V. A. A. (2016). Electrophoretic and biochemical characterization of *Tetragonisca weyrauchi* (Hymenoptera, Apidae) stingless bees esterase. *Scientia Agraria Paranaensis*, *15*(1), 70-75. DOI: https://doi.org/10.18188/1983-1471/sap. v15n1p70-75

Singh, B. N. (2016). The genus *Drosophila* is characterized by a large number of sibling species showing evolutionary significance. *Journal of Genetics*, *95*(4), 1053-1064. DOI: https://doi.org/10.1007/s12041-016-0699-x

Slobodian, V., Akama, A., & Dutra, G. M. (2017). A new species of *Pimelodella* (Siluriform: Heptapteridae) from the Guiana Shield, Brazil. *Zootaxa*, *4338*(1), 85-100. DOI: https://doi.org/10.11646/zootaxa.4338.1.4

Terra, M. C., Takagui, F. H., Baldissera, J. N. D. C., Lima, M. C. C., Feldberg, E., & Dias, A. L. (2022). The karyotype of *Pimelodella cristata* (Siluriform: Heptapteridae) from Central Amazon basin: with a discussion of the chromosome variability in *Pimelodella*. *Biologia*, *77*(7), 1-9. DOI: https://doi.org/10.1007/s11756-022-01056-0

Tonella, L. H., Ruaro, R., Daga, V. S., Garcia, D. A. Z., Vitorino Júnior, O. B., Magalhães, D. L., ... Lucena, Z. M. S. (2022). Neotropical freshwater fishes: A dataset of occurrence and abundance of freshwater fishes in the Neotropics. *Ecology*, *104*(4), 1-14. DOI: https://doi.org/10.1002/ecy.3713

Thorpe, J. P., & Solé-Cava, A. M. (1994). The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta*, *23*(1), 3-18. DOI: https://doi.org/10.1111/j.1463-6409.1994.tb00368.x

Vanhaecke, D., Leaniz, C. G., Gajardo, G., Young, K., Santana, J., Orellana, G., ... Consuegra, S. (2012). DNA barcoding and microsatellites help species delimitation and hybrid identification in endangered Galaxiid fishes. *PLoS ONE*, *7*(3),1-10. DOI: https://doi.org/10.1371/journal.pone.0032939

Ward, R. D., Skibinski, D. O. F., Woodwark, M. (1992). Protein heterozygosity, protein structure, and taxonomic differentiation. In M. K. Hecht (Ed.), *Evolutionary biology* (p. 73-159). New York, NY: Plenum Press.

Wright, S. (1949). The genetical estructure of populations. *Annals of Eugenics, 15*(1), 323-354. DOI: https://doi.org/10.1111/j.1469-1809.1949.tb02451.x

Yeh, F. C., Yang, R. C., Boyle, T. B. J., Ye, Z. H., & Mao, J. X. (1997). *Popgene, the user-friendly shareware for population genetic analysis, Molecular Biology and Biotechnology Center*. Edmonton, CA: University of Alberta.