



## Molecular genetics as a tool for fisheries management in the Brazilian Amazon: Piraíba (*Brachyplatystoma filamentosum* and *Brachyplatystoma capapretum*) (Siluriformes: Pimelodidae) in white-water rivers

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**Abstract.** Specimens from two fish species commonly known as piraíba (*Brachyplatystoma filamentosum* and *Brachyplatystoma capapretum*) were sampled from fish landings in nine white-water river channel regions of the Brazilian Amazon. We genetically characterized these specimens, through sequencing of the mitochondrial DNA control region. Of the 337 samples collected, 66.77% belonged to *B. filamentosum*, while the remaining 33.23% belonged to *B. capapretum*. For both species, the haplotype diversity (Hd) values were high (>0.95). However, the nucleotide diversity ( $\pi$ ) was higher in *B. filamentosum* (0.0118) than in *B. capapretum* (0.0041). We compared these values with the nucleotide diversity values of the control regions of other Amazonian fish species being overexploited or at risk of overexploitation.

**Keywords:** catfish, control region, filhote, fisheries genetics, mitochondrial DNA

**Resumo.** A genética molecular como ferramenta para o manejo da pesca na Amazônia brasileira: o caso da piraíba (*Brachyplatystoma filamentosum* and *Brachyplatystoma capapretum*) (Siluriformes: Pimelodidae) nos rios de águas brancas. Indivíduos de duas espécies de peixes popularmente conhecidas como piraíba *Brachyplatystoma filamentosum* e *Brachyplatystoma capapretum* oriundos dos desembarques pesqueiros realizados em nove regiões inseridas em calhas de rios de águas brancas da Amazônia brasileira, foram caracterizados geneticamente por meio do seqüenciamento da região controle do DNA mitocondrial. Para um total de 337 amostras, foi verificado que em média, na totalidade da área amostrada 66.77% dos peixes pertenciam à espécie *B. filamentosum* e 33.23% a espécie *B. capapretum*. Os valores de diversidade haplotípica foram altos em ambas as espécies; entretanto, a diversidade nucleotídica foi maior em *B. filamentosum* ( $\pi = 0.0118$ ) quando comparada a *B. capapretum* ( $\pi = 0.0041$ ). Esses valores foram comparados aos valores de diversidade nucleotídica da região controle de outras espécies de peixes sobreexploradas ou ameaçadas de sobreexploração na Amazônia.

**Palavras chave:** bagre, região controle, filhote, genética pesqueira, DNA mitocondrial

### Introduction

The Amazon Basin presents the greatest freshwater fish diversity in the world (estimated at more than 5000 species). However, fisheries activity in the region exploits approximately 100 species,

including seven migratory catfish species of the genus *Brachyplatystoma* (Siluriformes: Pimelodidae) (Barthem & Goulding 1997, Santos & Ferreira 1999, Santos *et al.* 2006). These can be characterized as species of considerable socioeconomic importance

(Carvalho & Barros 2008). Large catfish fisheries are located in Brazil, Peru, Colombia, and Bolivia. The Commission for Inland Fisheries of Latin America (COPESCAL) has emphasized the need to identify possible fish stocks (FAO 2000), in order to fulfil conservation and management actions (Vrijenhoek 1998; Ward 2000). Within the Amazon Basin, biological and fish production occurs more frequently in white-water rivers than in clear- and black-water rivers (Santos & Santos 2005).

The piraíba, *Brachyplatystoma filamentosum* (Lichtenstein), is considered the largest predator of Amazonian river channels. Frequently, its weight surpasses 100 kg. Piraíba smaller than 50 kg are also called filhote (Santos *et al.* 2006). Since the 1970s, the existence of one or more species of piraíba has been the subject of discussion (Goulding 1979). In 2005, *Brachyplatystoma capapretum*, known as dark piraíba, was described based on morphological characteristics (Lundberg & Akama 2005). In the present study, the term piraíba will be used collectively to refer to *B. filamentosum* and *B. capapretum*. It is possible to identify each species by differences in the premaxillary teeth. However, this is hindered by the practice of fish decapitation prior to landing. Furthermore, the fisheries and governmental sectors are not adapted to the new taxonomical review, and erroneously consider the two species as *B. filamentosum*. Based on samplings containing a mixture of *B. filamentosum* and *B. capapretum*, a knowledge base of the biology and population dynamics of piraíba has been constructed (Santos *et al.* 2006). The flesh is considered as premium for market purposes. However, differences in the migratory patterns and relative importance of *B. filamentosum* and *B. capapretum* for commercial fisheries production remain largely unknown (Araújo-Lima & Ruffino 2003, Barthem & Goulding 2007). Information regarding the geographical distribution of the two species indicates the presence of *B. filamentosum* in the entire extension of the Amazon Basin, whereas *B. capapretum* occurs in white- and black-water rivers at altitudes of <200 m above sea level (Barthem & Goulding 1997, Lundberg & Akama 2005).

More than 50% of piraíba landings in the Amazon occur in the frontier between Brazil, Peru, and Colombia. Of these, 90% are commercialized in the Colombian city of Letícia (close to the Brazilian city of Tabatinga). In this locality, the annual production of piraíba decreased from 4000 tons in 1983 to insignificant values in 2000, indicating overexploitation (Petrere *et al.* 2004, Barthem & Goulding 2007).

Tools such DNA barcoding, and sequencing of the control region and other mitochondrial genes,

have been used to detect population differences and clarify phylogenetic relationships in fish, and also to identify species and food products from different fisheries (Fraga *et al.* 2007, Gil 2007, Pappalardo *et al.* 2008, Teletchea 2009). In the present study, we used sequencing analysis of the DNA mitochondrial control region to estimate the frequency of occurrence and genetic variability of *B. filamentosum* and *B. capapretum*, among piraíba landed by commercial fisheries located in white-water rivers of the Brazilian Amazon.

## Materials and Methods

### *Sample collection and mitochondrial DNA control region sequencing*

A total of 337 samples of muscular tissue or fins of fish specimens, commercialized using the common name piraíba, were collected from 14 fish-landing and commercial fishery localities along the white-water river channel of the Brazilian Amazon Basin between 1998 and 2008. The localities sampled were distributed in nine regions characterized by sub-basins, referred to by their location in relation to the main axis of the River Solimões/Amazon (Figure 1, Table I).

The samplings were carried out as part of the 'Subsidies for the management and conservation of the large migratory catfish in the Amazon and for the identification and genetic characterization of the fishery stock' project, by the National Institute for Amazonian Research (INPA). Forty-eight samplings were performed at different intervals, in accordance with the logistics necessary for the vast geographical extension of the Amazon Basin. The samplings were authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), through process number 02005.001811/04-65.

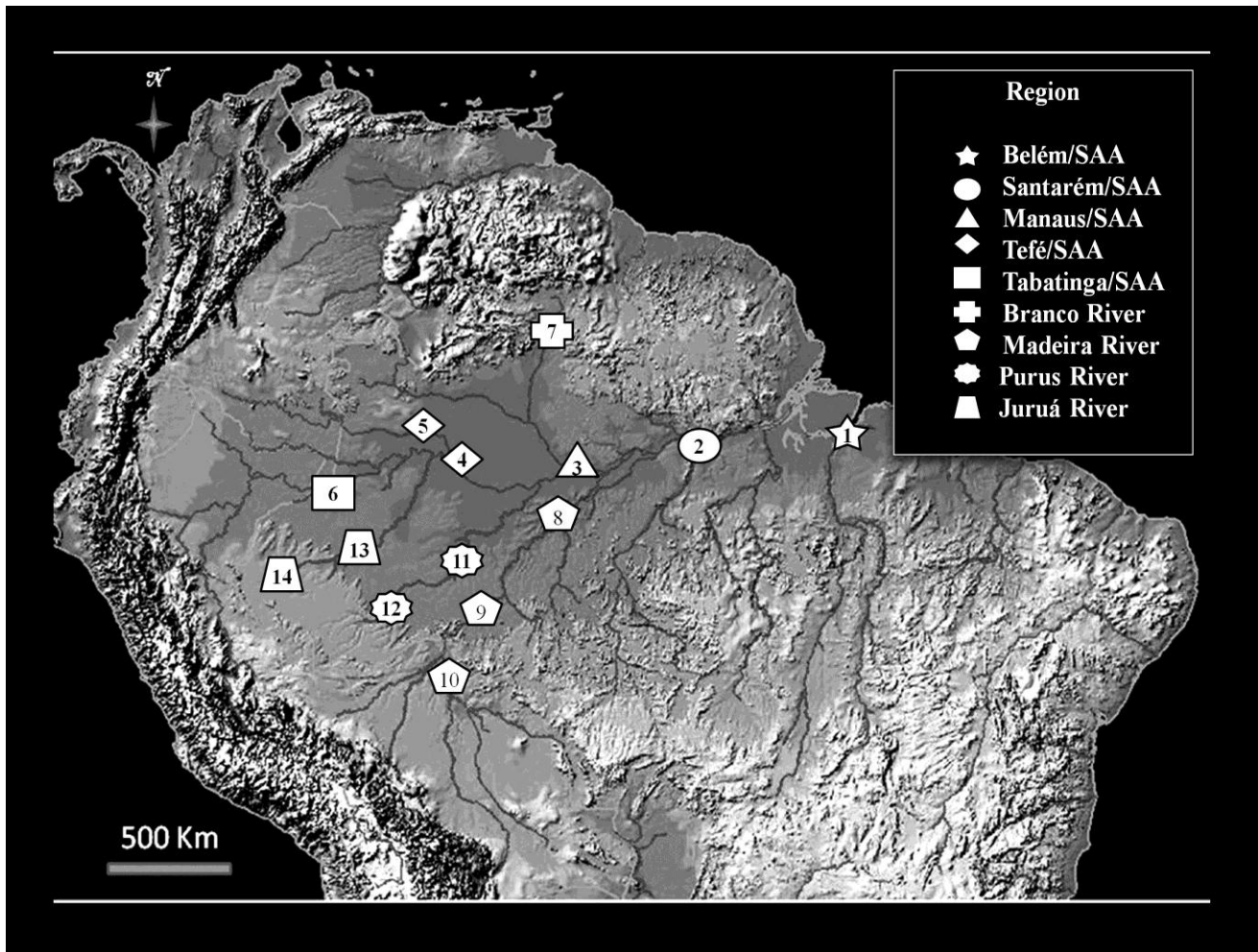
The samples were conserved in 90% ethanol at -80°C. Total DNA extraction, amplification, and complete sequencing of the mitochondrial DNA control region were performed following the procedures described by Batista and Alves-Gomes (2006) with modifications. We designed two primers for PCR (FTTP-L 5'- CCA AGC GCC GGT CTT GTA A - 3', and DLR1-H 5' - GGA TAC TTG CAT GTA TAA ATT GG - 3'). Primers FTTP-L and F-12R (5'- GTC AGG ACC ATG CCT TTG TG - 3') (Sivasundar *et al.* 2001) were used for sequencing.

### *Species identification and sequence alignment*

Six reference sequences (three for each species) were obtained from the fish specimen samples morphologically categorized as *B. filamentosum* or *B. capapretum* (Lundberg & Akama 2005). For each species, the sequences were aligned using BioEdit 7.0.9.0 software (Hall 1999), and

assembled in a matrix. The final alignment between the two species was performed through the insertion of five gaps in *B. filamentosum* and six gaps in *B.*

*capapretum*, resulting in a matrix of 919 nucleotide sites.



**Figure 1.** Sampled localities and their respective assemblages per region, indicated by the shape of the markers: (1) Belém; (2) Santarém; (3) Manaus; (4) Tefé; (5) Japurá; (6) Tabatinga; (7) Boa Vista; (8) Manicoré; (9) Porto Velho; (10) Guajará Mirim; (11) Lábrea; (12) Boca do Acre; (13) Eirunepé; and (14) Cruzeiro do Sul. SAA = Solimões/Amazon axis.

The identification of the remaining 331 DNA sequences was performed through alignment with the reference sequences and by comparison with 60 nucleotide sites characteristic of each species (Table II).

The sequences were deposited into the GenBank (*B. filamentosum*: accession numbers between GU441886 and GU442086, and between GU442098 and GU442121; and *B. capapretum*: accession numbers between GU903349 and GU903460).

*Frequency of occurrence of Brachyplatystoma filamentosum and Brachyplatystoma capapretum among piraíba captured by commercial fisheries*

After identifying the number of specimens of each species for each sampled locality, the specimens were assembled according to region. The mean frequency of each species, taking into account all regions, was estimated as the mean frequency of each species captured by commercial fisheries in white-water rivers of the Brazilian Amazon.

#### *Measurement of furcal lengths*

The furcal lengths were measured in 242 fish specimens (164 of *B. filamentosum* and 78 of *B. capapretum*), which were decapitated and gutted. The mean furcal lengths were compared using the *t*-test ( $\alpha = 0.05$ ).

*Genetic diversity and demographic analyses*

Using Arlequin 3.11 software (Excoffier *et al.* 2005), we estimated the following genetic parameters for the mitochondrial DNA control region of each species: number of haplotypes; number of singletons; haplotype diversity (Hd); probability of distinction of both sequences in a

population) (Nei 1987); nucleotide diversity ( $\pi$ : number of nucleotide differences per site between the sequences) (Nei 1987); total number of nucleotide sites; number of polymorphic sites (S); number of transitions (TS); number of transversions (TV); number of indels (insertion/deletion events); and nucleotide composition.

**Table I.** Regions, respective sampling localities, and number of samples of piraíba (*Brachyplatystoma sp.*).

Region	Localities	State	Geographical coordinates	Number of samples of piraíba
Belém (SAA)	Belém	Pará	01°27'18.04"S 48°30'08.90"W	36
Santarém (SAA)	Santarém	Pará	02°26'25.58"S 54°41'54.70"W	42
Manaus (SAA)	Manaus	Amazonas	03°05'39.60"S 60°01'33.63"W	28
Tefé (SAA)	Tefé	Amazonas	03°18'22.60"S 64°45'33.50"W	18
	Japurá	Amazonas	01°49'34.81"S 66°35'58.32"W	64
Tabatinga (SAA)	Tabatinga	Amazonas	04°15'11.62"S 69°56'19.36"W	25
Branco River	Boa Vista	Roraima	02°50'23.75"N 60°39'43.61"W	21
Madeira River	Porto Velho	Rondônia	08°45'42.85"S 63°54'06.77"W	51
	Guajará Mirim	Rondônia	10°47'27.28"S 65°19'56.74"W	04
	Manicoré	Amazonas	05°48'33.77"S 61°18'01.77"W	02
Purus River	Lábrea	Amazonas	07°12'20.72"S 64°50'36.99"W	30
	Boca do Acre	Amazonas	08°43'45.81"S 67°24'29.67"W	03
Juruá River	Cruzeiro do Sul	Acre	07°38'10.54"S 72°40'02.86"W	05
	Eirunepé	Amazonas	06°37'40.87"S 69°54'04.24"W	08
Total				337

SAA = Solimões/Amazon axis.

The demographic dynamics of each species were inferred by two methods. The first of these was Fu's test for hypothesis of mutational neutrality ( $F_s$ ) with statistical significance conditioned to 10000 permutations (Fu 1997). This test considers that there is no selective advantage between haplotypes, and that significant mutational unbalance is associated with recent population expansion or a bottleneck effect. The second method was the analysis of mismatch distribution (pairwise nucleotide differences) (Rogers & Harpending

1992), which is bimodal on stable populations and unimodal on populations that endure sudden expansion. Both procedures were carried using Arlequin 3.11 software (Excoffier *et al.* 2005).

## Results

*Frequency of occurrence of Brachyplatystoma filamentosum and Brachyplatystoma capapretum among piraíba captured by commercial fisheries*

From the 337 piraíba samples analysed, 225 were found to have *B. filamentosum* and 112 had *B.*

*capapretum*. Figure 2 shows the frequency of each species per region, and the estimated mean frequency of each species among the pirafiba captured by commercial fisheries in the white-water

rivers of the Brazilian Amazon. With the exception of Manaus and the Purus River, the frequency of *B. filamentosum* was consistently higher than that of *B. capapretum*.

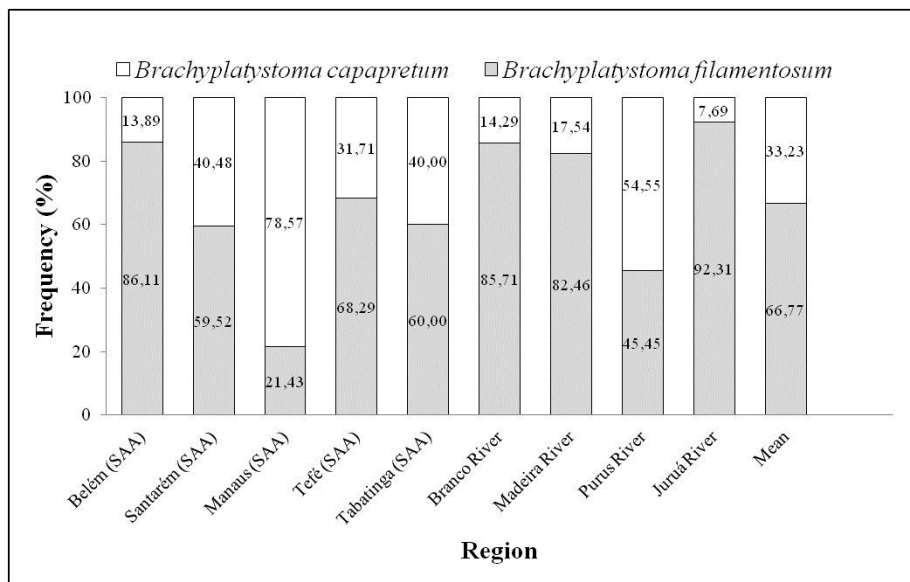
**Table II.** Nucleotide sites of the mitochondrial DNA control regions of *Brachyplatystoma filamentosum* and *Brachyplatystoma capapretum* used in the identification of the sequences and their respective species.

GenBank accession number	Species	Locality	Nucleotide position (5'→3')																																
			0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3		
GU441891	<i>Brachyplatystoma filamentosum</i>	Belém	T	T	T	T	T	G	C	C	G	A	C	C	A	C	C	A	A	A	C	C	C	C	C	C	C	C	G	A	A	C	T		
GU441892	<i>Brachyplatystoma filamentosum</i>	Belém	T	T	T	T	T	G	C	C	G	A	C	C	A	C	C	A	A	A	C	C	C	C	C	C	C	G	A	A	C	T			
GU441895	<i>Brachyplatystoma filamentosum</i>	Belém	T	T	T	T	T	G	C	C	G	A	C	C	A	C	C	A	A	A	C	C	C	C	C	C	C	G	A	A	C	T			
GU903353	<i>Brachyplatystoma capapretum</i>	Belém	C	C	C	C	C	A	T	G	T	G	T	T	G	T	T	G	T	C	T	T	A	T	T	T	A	G	T	T	A				
GU903349	<i>Brachyplatystoma capapretum</i>	Belém	C	C	C	C	C	A	T	G	T	G	T	T	G	T	T	G	T	C	T	T	A	T	T	T	A	G	T	T	A				
GU903350	<i>Brachyplatystoma capapretum</i>	Belém	C	C	C	C	C	A	T	G	T	G	T	T	G	T	T	G	T	C	T	T	A	T	T	T	A	G	T	T	A				
			3	3	3	3	4	4	4	4	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	8	8	8
			3	3	3	6	3	5	5	5	4	6	9	0	0	0	2	4	5	6	6	7	8	0	1	2	4	5	5	0	2	3			
			3	4	8	9	4	4	5	6	9	5	0	5	8	9	8	8	4	7	8	2	6	9	7	7	3	0	1	0	2	1			
GU441891	<i>Brachyplatystoma filamentosum</i>	Belém	A	T	C	C	G	G	C	C	T	T	A	A	G	A	A	A	A	A	T	A	T	C	C	A	T	G	C	C	C	T			
GU441892	<i>Brachyplatystoma filamentosum</i>	Belém	A	T	C	C	G	G	C	C	T	T	A	A	G	A	A	A	A	A	T	A	T	C	C	A	T	G	C	C	C	T			
GU441895	<i>Brachyplatystoma filamentosum</i>	Belém	A	T	C	C	G	G	C	C	T	T	A	A	G	A	A	A	A	A	T	A	T	C	C	A	T	G	C	C	C	T			
GU903353	<i>Brachyplatystoma capapretum</i>	Belém	G	C	T	T	A	C	A	T	C	C	G	G	A	T	G	G	G	T	C	G	C	T	T	G	C	A	T	T	T	C			
GU903349	<i>Brachyplatystoma capapretum</i>	Belém	G	C	T	T	A	C	A	T	C	C	G	G	A	T	G	G	G	T	C	G	C	T	T	G	C	A	T	T	T	C			
GU903350	<i>Brachyplatystoma capapretum</i>	Belém	G	C	T	T	A	C	A	T	C	C	G	G	A	T	G	G	G	T	C	G	C	T	T	G	C	A	T	T	T	C			

*Measurement of furcal lengths*

The mean (± s. d.) furcal length was 88.23 (± 25.75) cm for *B. filamentosum* and 71.35 ±

(23.01) cm for *B. capapretum*. The *t*-test revealed no significant statistical difference between the two species (*p* > 0.05).



**Figure 2.** Frequency of *Brachyplatystoma filamentosum* and *Brachyplatystoma capapretum* per region and estimated mean frequency of each species among pirafiba captured by commercial fisheries in the white-water rivers of the Brazilian Amazon. SAA = Solimões/Amazon axis.

*Genetic diversity and demographic analyses*

The estimates of the genetic parameters for the mitochondrial DNA control region of *B.*

*filamentosum* and *B. capapretum* are presented in Table III. The values for haplotype diversity are similar for both species. By contrast, the values for

nucleotide diversity ( $\pi$ ) and number of polymorphic sites (S) indicate a smaller number of substitutions of the mitochondrial control region in *B. capapretum* than in *B. filamentosum*.

**Table III.** Estimates of the genetic parameters for the mitochondrial DNA control region of *Brachyplatystoma filamentosum* and *Brachyplatystoma capapretum*.

Genetic parameters		<i>Brachyplatystoma filamentosum</i>	<i>Brachyplatystoma capapretum</i>
Number of samples		225	112
Number of haplotypes		130	53
Number of singletons		96	40
Haplotype diversity (Hd $\pm$ s.d.)		0.9871 $\pm$ 0.0028	0.9509 $\pm$ 0.0106
Nucleotide diversity ( $\pi$ $\pm$ s.d.)		0.0118 $\pm$ 0.0060	0.0041 $\pm$ 0.0023
Total number of nucleotide sites		914	913
Number of polymorphic sites (S)		68	34
Number of transitions (TS)		63	27
Number of transversions (TV)		06	03
Number of indels		02	04
Nucleotide composition	A	32.72%	31.65%
	C	21.86%	20.72%
	G	13.75%	14.60%
	T	31.67%	33.02%

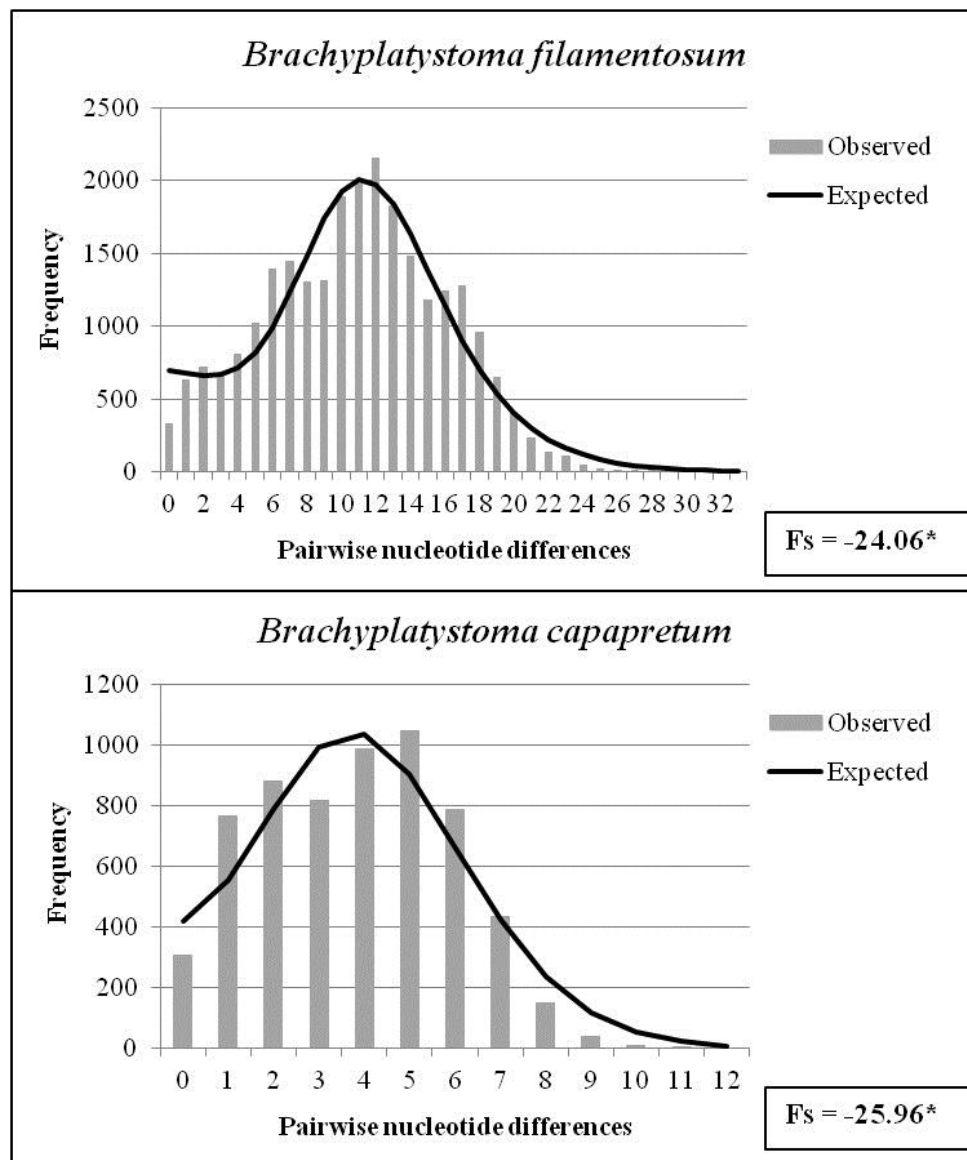
Fu's test demonstrated a statistically significant deviation of mutational neutrality for *B. filamentosum* and *B. capapretum* (Figure 3). Thus, our genetic diversity and demographic analyses indicated either a sudden expansion of populations, or a bottleneck effect, to which both species were previously submitted. The shape of the mismatch distribution was unimodal for both species, corroborating the possibility of sudden population expansion (Figure 3).

## Discussion

The mitochondrial DNA has previously been shown to be a very efficient molecular marker for identifying fish species captured in the rivers of the Brazilian Amazon (Ardura *et al.* 2010). Use of the control region successfully inferred the phylogeography of the dourada, *Brachyplatystoma rousseauxii* (Castelnau) (Batista & Alves-Gomes 2006), and also the panmixia of the piramutaba, *Brachyplatystoma vaillantii* (Valenciennes) (Formiga-Aquino 2004), and the tambaqui, *Colossoma macropomum* (Cuvier) (Santos *et al.* 2007), along the River Solimões/Amazon.

The nucleotide compositions of *B. filamentosum* and *B. capapretum* determined in the present study are similar to those of the control regions for other mitochondrial genomes, with a

predominance of A and T bases (Chiang *et al.* 2006). Based on the relationship proposed by Grant and Bowen (1998) between haplotype and nucleotide diversities of marine fish mitochondrial DNA, we can classify *B. filamentosum* as a species that presents a large stable population, with a long history of evolution or secondary contact among different lineages; in contrast, *B. capapretum* is a species that has suffered a previous bottleneck effect, followed by rapid population growth. For the acoupa weakfish *Cynoscion acoupa* (Lacepède), a demersal marine species landed in the Amazon estuary, the mean nucleotide diversity of the mitochondrial DNA control region ( $\pi = 0.0028$ ) was considered low, probably as a result of overexploitation (Rodrigues *et al.* 2008). By contrast, for the tambaqui (*C. macropomum*), which suffers strong fishing pressure in the Amazon Basin, the mean nucleotide diversity ( $\pi = 0.0120$ ) of the mitochondrial DNA control region was considered high (Santos *et al.* 2007). Similar nucleotide diversity values of the mitochondrial DNA control region were observed for two heavily exploited Amazonian catfish, the dourada (*B. rousseauxii*;  $\pi = 0.0095$ ) (Batista & Alves-Gomes 2006) and the piramutaba (*B. vaillantii*;  $\pi = 0.0166$ ) (Formiga-Aquino 2004).



**Figure 3.** Estimates of the values of mutational neutrality ( $F_s$ ) of Fu's test and histograms of the mismatch distribution for both species with the observed and expected values (\* $p < 0.05$ ).

Our present findings indicate that *B. capapretum*, in addition to presenting a smaller effective population size, may have suffered a previous bottleneck effect caused by overexploitation. The reasons for the more acute overexploitation of *B. capapretum* compared with *B. filamentosum* remain unclear. The mismatch distribution indicates that both species have previously undergone sudden demographic expansions, and this creates uncertainty regarding the relationship between fishing and the decrease in genetic variability exhibited by *B. capapretum*. Using mitochondrial DNA restriction fragment length polymorphism (RFLP) of cytochrome b and the control region, Coronel *et al.* (2004) suggested that *B. rousseauxii* may have suffered a historical

bottleneck effect in the Bolivian Amazon. The contraction–expansion dynamics of fish populations may also be associated with events unrelated to the fishing, such environmental variations during the Pleistocene (Atarhouch *et al.* 2006, Hubert *et al.* 2007). Bouzat (2000) recommended that, to avoid the incurrence of interpretative errors, inferences regarding the relationship between genetic diversity and effective population size should be accompanied by measures of genetic diversity to serve as a temporal control.

The piraba is generally captured using two types of fishing gear: longlines and gillnets. The lack of distinction between *B. filamentosum* and *B. capapretum* means that there is no preference or price difference for either type of flesh. Thus,

assuming that the same type of fishing gear and effort were applied when capturing the fish sampled in the present study, it is unlikely that the frequency of each species per region were influenced by these factors. Although *B. filamentosum* predominated in most of the sampled regions, *B. capapretum* was predominant in Manaus and the Purus River. This variation may reflect the timing of sampling in relation to migration, which is likely to differ between the two species. However, other factors contributing to the different frequency patterns of the two species remain to be determined. Further studies are required to clarify the migratory pattern of each species and the relationship with the proportions landed, in conjunction with other research tools such as biotelemetry and population dynamics. The piraíba migrates longitudinally towards the headwaters of rivers (Agudelo-Córdoba *et al.* 2000). In common with other Amazonian fish species, such behaviour is closely related to the flood pulse of the basin (Santos & Jegu 2004). In the Colombian Amazon, the reproductive period of the piraíba occurs in the drawdown waters, and the first reproductive events probably begin between 6 and 10 years of age (Agudelo-Córdoba *et al.* 2000).

By adjusting the mean frequency of each species documented in the official fisheries statistics published by IBAMA between 2001 and 2007 (Ruffino *et al.* 2002, 2005, 2006, IBAMA 2007a, b, 2008, Thomé-Souza *et al.* 2007), we estimate that approximately 2575 tons of *B. capapretum* (33% of the total captured piraíba) were commercialized as *B. filamentosum* in the Brazilian states of Amazonas and Pará.

According to the normative statement number five, on 21<sup>st</sup> May 2004, prepared by the Environmental Ministry of Brazil, the tambaqui (*C. macropomum*), dourada (*B. rousseauxii*), and piramutaba (*B. vaillantii*) are included in the official list of fish being overexploited or at risk of overexploitation. This normative statement is a legal instrument of the Brazilian Government, prepared in accordance with the Convention on Biological Diversity (CBD) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora in Danger of Extinction (CITES). Its purpose is to stimulate the creation of management plans that address the recovery of stocks and sustainability of fisheries. *B. filamentosum* and *B. capapretum* are not included in the list. The creation of policies aimed at fisheries management of the piraíba will become extremely difficult in the near future, mainly because of the complex fisheries landscape, which includes considerable socioeconomic and cultural diversities, the substantial geographical extension of the Amazon Basin, and the vast number of people

involved in artisanal “diffuse” fisheries (Ruffino 2008). Moreover, the problem is aggravated by the construction of power plants in the Amazonian rivers. The construction of these power plants will have a negative impact on the life cycle of migratory species (Barthem *et al.* 1991). In addition to prioritizing fisheries production and biodiversity maintenance, fisheries management must support actions that promote the integrity of areas important to the life cycle of the species, including constant follow-ups and the involvement of fishermen (Agostinho *et al.* 2005). Given that the life cycle of large catfish species incorporates spawning and growth regions in different South American countries (Barthem & Goulding 1997), fishing negotiations and international agreements are essential for their conservation.

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