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Original Article

Reduced genetic diversity and the success of the invasive peacock bass (Cichliformes: Cichlidae)

Diversidade genética reduzida e o sucesso da espécie invasora tucunaré (Cichliformes: Cichlidae)

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Abstract

Several species of *Cichla* successfully colonized lakes and reservoirs of Brazil, since the 1960's, causing serious damage to local wildlife. In this study, 135 peacock bass were collected in a reservoir complex in order to identify if they represented a single dominant species or multiple ones, as several *Cichla* species have been reported in the basin. Specimens were identified by color pattern, morphometric and meristic data, and using mitochondrial markers COI, 16S rDNA and Control Region (CR). Overlapping morphological data and similar coloration patterns prevented their identification using the taxonomic keys to species identification available in the literature. However, Bayesian and maximum likelihood from sequencing data demonstrated the occurrence of a single species, *Cichla kelberi*. A single haplotype was observed for the 16S and CR, while three were detected for COI, with a dominant haplotype present in 98.5% of the samples. The extreme low diversity of the transplanted *C. kelberi* evidenced a limited number of founding maternal lineages. The success of this colonization seems to rely mainly on abiotic factors, such as increased water transparency of lentic environments that favor visual predators that along with the absence of predators, have made *C. kelberi* a successful invader of these reservoirs.

Keywords: cichlid, low genetic diversity, bottleneck, transplanted species, top predator.

Resumo

Muitas espécies de *Cichla* colonizaram com sucesso lagos e reservatórios do Brasil desde os anos 1960, causando graves prejuízos à vida selvagem nesses locais. Neste estudo, 135 tucunarés foram coletados em um complexo de reservatórios a fim de identificar se representavam uma espécie dominante ou múltiplas espécies, uma vez que diversas espécies de *Cichla* foram registradas na bacia. Os espécimes foram identificados com base na coloração, dados morfométricos e merísticos, e por marcadores mitocondriais COI, 16S rDNA e Região Controle (RC). A sobreposição dos dados morfométricos e o padrão similar de coloração impediram a identificação utilizando as chaves de identificação disponíveis na literatura. Entretanto, as análises bayesiana e de máxima verossimilhança de dados moleculares demonstraram a ocorrência de uma única espécie, *Cichla kelberi*. Um único haplótipo foi observado para o 16S e RC, enquanto três foram detectados para o COI, com um haplótipo dominante presente em 98,5% das amostras. A baixa diversidade nos exemplares introduzidos de *C. kelberi* evidenciou um número limitado de linhagens maternas fundadoras. O sucesso da invasão parece depender de fatores abióticos, como a maior transparência da água de ambientes lênticos que favorece predadores visuais que, atrelado à ausência de predadores, fez do *C. kelberi* um invasor bem-sucedido nesses reservatórios.

Palavras-chave: ciclídeo, baixa diversidade genética, efeito gargalo, espécies introduzidas, predador de topo.

1. Introduction

Peacock basses (genus *Cichla*; Cichliformes: Cichlidae) are native to the Amazon, Tocantins and Orinoco basins, as well as Atlantic-slope rivers of the Guianas and Suriname (Kullander and Ferreira, 2006). They are non-migratory carnivorous fish, with parental care, as they build nests and protect their offspring (Gomiero et al., 2009). They are among the most frequently introduced species in Brazil for sport fishing in reservoirs, lakes and rivers (Gomiero and

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Braga, 2003; Ferrareze and Nogueira, 2015; Marques et al., 2016), as also in North America, Africa and Asia (Golani et al., 2019; Sastraprawira et al., 2020), and represent a potential threat to the native fish fauna.

The introduction of species causes problems related to the dissemination of diseases and/or parasites and predator-prey interactions. For example, invasions of *Cichla* spp. in rivers and lakes of Panama have drastically reduced fish assemblages (Zaret and Paine, 1973), from which they have not yet recovered (even after 45 years) (Sharpe et al., 2017), and has been associated to the extinction of native species in the Atlantic Forest of Brazil (Pelicice and Agostinho, 2009; Fragoso-Moura et al., 2016). In invasions, aspects such as history of successful colonization, high physiological tolerance, an ability to deal with anthropogenic impact on a habitat, similar conditions between source and recipient environment, and large propagule size (>100 individuals) have been described as key factors used to predict successful invasions (Moyle and Marchetti, 2006).

In the case of peacock bass invasions in reservoirs, environmental factors seem to favor this diurnal visual predator. Espínola et al. (2010) compared 63 Brazilian reservoirs with records of presence/absence of *Cichla*, and corroborated that the deepest, most transparent and warmest reservoirs were the most colonized ones. Later, Franco et al. (2018) examined other 12 Brazilian reservoirs and concluded that the abundance of *Cichla* was associated with warm temperatures and low turbidity, what is due to increased water residence time.

One of the primordial aspects when assessing peacock bass colonization is the taxonomic identity of the invaders, i.e., if there is one dominant species or a group of them. The genus *Cichla* shows an extensive phenotypic variation, especially related to the color pattern, which can be very confusing for species identification (Reiss et al., 2012; Quadros et al., 2020). The misidentification of several *Cichla* species introduced in different regions of Brazil was previously reported by Kullander and Ferreira (2006), such as for *C. kelberi* (Kullander & Ferreira, 2006), which was erroneously identified as *C. monoculus* Spix & Agassiz, 1831 and *C. piquiti* (Kullander & Ferreira, 2006), due to the overlapping morphometric characters and similar coloration patterns. Molecular data could be a useful complement for more conclusive identification in complex genus, such as *Cichla*, due to conflicting morphometric and meristic characteristics.

Four peacock bass species (*C. kelberi*, *C. piquiti*, *C. temensis* (Humboldt, 1821), and *C. monoculus*) have reportedly been introduced into lakes, reservoirs, tributaries, and the main channel of the São Francisco River Basin (Pompeu and Godinho, 2003; Sato and Sampaio, 2005; Carvalho et al., 2009). However, due to the different morphotypes found in this river system, it is unclear whether they represent multiple species or a single one.

In this context, molecular data could be a useful complement for more conclusive identification in complex genus, such as *Cichla*, due to conflicting morphometric and meristic characteristics. The use of molecular tools as a method of taxonomic identification of closely related species has been previously reported (Carvalho et al., 2009; Hashimoto et al., 2016). Mitochondrial markers such as cytochrome c oxidase subunit I (COI), 16S ribosomal DNA, and control region (CR), are widely used as molecular tools to aid in the identification of freshwater fish species (Cheng et al., 2012; Pereira et al., 2013; Quraishia et al., 2015; Saad, 2019).

The purpose of this study was to determine if one or more species of *Cichla* have been introduced into reservoirs of the basin using morphometric and genetic data.

2. Material and Methods

2.1. Ethics statement

All biological material used in this research came from artisanal fishery and data were collected at landing ports. No live specimen was kept in captivity or manipulated. Therefore, no ethical approval was necessary.

2.2. Fish sampling and identification

The samples were obtained after the fish were caught by artisanal fishermen, at landing sites in three reservoirs, Moxotó, Delmiro Gouveia and PA IV, located in the submiddle stretch of the São Francisco River (Figure 1).

Species identification was based on coloration, meristics, and morphological characteristics including presence/absence or placement of bars and spots on the body, lateral line scale counts and background side coloration, according to Kullander and Ferreira (2006). The taxonomical and morphometric analysis was performed on 113 specimens (77 females and 36 males), selected according to their morphological integrity such as undamaged fins and complete scale cover. Nine body measurements were taken using a digital caliper (0.1 mm), and 11 meristic characters were counted (Table 1) for taxonomical identification, following Kullander and Ferreira (2006). Body depth, caudal peduncle height, head depth, eye diameter and interorbital distance (expressed as proportions of standard length (SL)), along with meristic data and color were compared with those presented by Kullander and Ferreira (2006) for the four species of *Cichla* reported for the São Francisco basin. These authors described the living coloration on the side of *C. kelberi, C. piquiti, C. monoculus* and *C. temensis*, collected in naturally occurring basins, as "pale grey", "greyish", "dull olivaceous", "yellowish" and "golden".

2.3. Molecular analysis

Based on extensive molecular data, Willis et al. (2012) considered only eight species: *C. orinocensis*, *C. intermed*ia, C. *ocellaris*, *C. temensis, C. melaniae, C. mirianae, C. piquiti,* and *C. pinima* in the genus *Cichla,* instead of the 15 described by Kullander and Ferreira (2006). The *C. monoculus*, *C. kelberi*, *C. nigromaculata* and *C. pleiozona* were classified as subspecies of *C. ocellaris sensu lato*. In this study, *C. kelberi* and *C. monoculus* were considered as valid species, as most of the sequences of *Cichla* deposited in the GenBank followed Kullander and Ferreira (2006) classification.

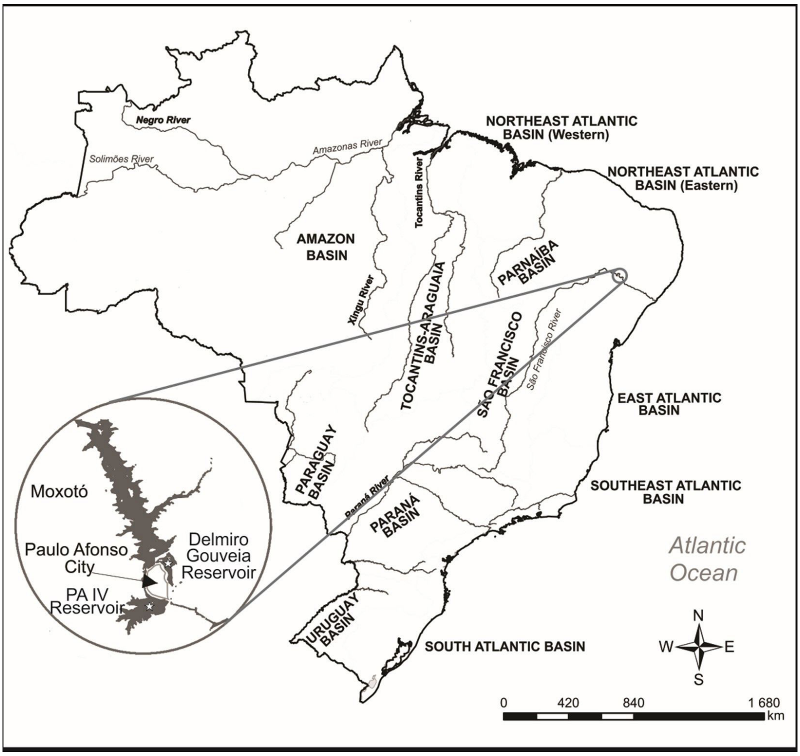

Figure 1. Brazilian hydrographic basins and sampling area in detail.

Table 1. Description of morphometric measurements and meristic data used.

Meristic data		Longitudinal morphometric data	
1st DS	1st dorsal fin spine number	SL	Standard length
2nd DS	2nd dorsal fin spine number	BD	Body depth
2nd DR	2nd dorsal fin ray number	CPH	Caudal peduncle height
CFR	Caudal fin ray number	HD	Head depth
AFR	Anal fin ray number	ED	Eye diameter
VFR	Ventral fin ray number	ID	Interorbital distance
PFRS	Pectoral fin ray and spine number	PDD	Predorsal distance
CPS	Caudal peduncle scale number	PVD	Preventral distance
ALL	Anterior lateral line scale number	PAD	Preanal distance
PLL	Posterior lateral line scale number		
MLL	Median lateral line scale number		

For molecular analysis, muscle samples from 135 specimens were collected from the caudal peduncle of each specimen and preserved in 95% ethanol for subsequent extraction of genetic material. Additionally, four muscle samples (from naturally occurring basins) of *C. piquiti* (CP), identified as *C. piquiti* 01, *C. piquiti* 02, *C. piquiti* 03, and *C. piquiti* 05, and two muscle samples of *C. kelberi*, identified as *C. kelberi* 06 and *C. kelberi* 07, were collected from Tocantins River (TO) by Prof. Alberto Akama from the Museum Emílio Goeldi, Belém, Brazil. Furthermore, two muscle samples of *C. monoculus* from Xingu River, *C. monoculus* 02 and *C. monoculus* 03 (Xingu Project), and two muscle samples of *C. temensis* (*C. temensis* 01 and *C. temensis* 02) from the Negro River (SUDAM – Proc. nº CUP 59004/00473/2013-42) were all sequenced.

Total DNA was extracted using the modified phenol-chloroform-isoamyl alcohol method (Sambrook et al., 1989). Three mitochondrial markers were amplified: Cytochrome c Oxidase subunit I (COI), 16S ribosomal DNA (16S), and Control Region (CR). Polymerase chain reaction (PCR) amplification of COI was performed with the primers BarcFish11 and BarcFish2, as described by Ward et al. (2005). Part of the 16S and CR were amplified with the primers 16S-L1987 and 16S-H2909 as described by Palumbi et al. (1991), and L (Cronin et al., 1993), and H16498 (Meyer et al., 1990), respectively. Amplicons of 650 bp (COI), 500 bp (16S) and 460 bp (CR) were purified with the enzymes ExoI/SAP and sequenced using a Genetic Analyzer (3500 Applied Biosystems, CA, USA).

2.4. Data analysis

The morphometric data were linearized in order to achieve a normal variance distribution, and transformed to eliminate allometric effects (Rohlf, 1990), using the equation $Y^*_i = Y_i\ (X_0/X_i)^{\beta}$, which corrected size effects; where Y^*_i = standardized morphometric measurement for each individual i; Y_i = i-th morphometric measurement Y; X_0 = average standard length (SL) of all individuals; X_i = individual standard length i; and β = allometric coefficient from the linearized equation $\log Yi = \log\alpha + \beta \log Xi$, using the entire sample.

All morphometric data were analyzed for normality and homoscedasticity. Since they did not meet the parametric requirements, the non-parametric Kruskal-Wallis test was conducted in order to evaluate differences among types and the other four *Cichla* species reported for this river system. All analyses were made using Statistica 6.0.

The DNA sequences were edited and aligned in the MEGA 7 program (Kumar et al., 2016), using ClustalW (Thompson et al., 1994). After trimming the poor-quality regions, final alignments of 628 bp (COI), 408 bp (16S) and 358 bp (CR) were obtained. The nucleotide sequences generated in this study were deposited in GenBank under the accession numbers MW248167-MW248311 (COI), MW255385-MW255471 (16S), and MW251997-MW252032 (CR). GenBank sequences, whose specimens were taken from the naturally occurring basins of each species, were selected for subsequent comparisons. For 16S (*C. kelberi* FJ904290- Tocantins River; *C. monoculus* FJ904288- Solimões River and AF049017- Negro River; *C. piquiti* FJ904286- Tocantins River and *C. temensis* AF049019- Negro River; and for CR (*C. kelberi* JQ926871-JQ926872, FJ890808, FJ890812, FJ890813, GU295705-GU295707; *C. monoculus* GU295709-GU295732 and DQ841872-DQ841899; *C. piquiti* JQ926783-JQ926792 and *C. temensis* GU295739-GU295740 and DQ841909-DQ841929), all from different locations from the Amazonas, Orinoco, Essequibo, Maroni and Tocantins Rivers. In the phylogenetic trees, GenBank sequences were named by a combination of the species names (*C. monoculus, C. kelberi, C. temensis* and *C. piquiti*), followed by the last three digits of their accession numbers.

Phylogenetic analyses for each marker were carried out with Maximum Likelihood (ML) and Bayesian Inference (BI). The best-fit model (COI = HKY+I, 16S = TIM2+I and CR= HKY+I) of nucleotide substitution was identified using jModelTest (Posada, 2008). The selected model was implemented into PhyML 3.0 (Guindon and Gascuel, 2003) to perform ML reconstruction using the approximate likelihood ratio test to evaluate node support. Additionally, a probabilistic topology was obtained through Bayesian inference (BI). The BI analysis was conducted in MrBayes v.3.0b4 (Ronquist and Huelsenbeck, 2003) using 2,000,000 generations, with sampling every 100 generations, and a burn-in of 25%. The default values of the program were used for the other variables. Support for the branches of the BI phylogenies was estimated with posterior probability (Huelsenbeck and Ronquist, 2001). *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (KU531434) was used as an outgroup for the three markers, considering recent phylogenetic evidence (López-Fernández et al., 2010).

The distance model Kimura 2-parameter (K2P) was used to estimate the overall mean genetic distance for all the COI samples, as implemented in MEGA 7 (Kimura, 1980; Kumar et al., 2016). Estimation of haplotypes number, frequencies of haplotypes, nucleotide and haplotype diversity, were performed using DNAsp v.5.10.00 software (Librado and Rozas, 2009). The haplotype network was determined using the Network 4.5.6 program and calculated using the median-joining algorithm (Bandelt et al., 1999).

3. Results

The morphometric and meristics data overlapped between the four reported species for the basin and the sampled individuals (Table 2), as well as the presence of a conflicting pigmentation pattern in the latter made their taxonomic identification unclear. Two distinct patterns of color and pigmentation were identified in the sampled individuals, what might suggest the presence of more than one species. Therefore, they were preliminarily classified into “gray-greenish” and “yellowish” types, considering the predominant (>50%) background side color (Figure 2).

Out of the 113 individuals included in the morphometric analysis, 46 (SL=170-293 mm) belonged to the “gray-greenish” type, and 67 (SL=200-313 mm) to the “yellowish” type (Figure 2). The five body proportions were analyzed for differences between the two color types, and compared with the data for *Cichla monoculus, C. kelberi, C. piquiti* and *C. temensis* (Table 2). No significant differences were found for any of the body proportions analyzed between types alone, nor between types and the four *Cichla* species. Also, the counts of meristic data overlapped between the four *Cichla* species (Table 2).

Peacock bass specimens exhibited two color types and considerable morphometric variation, including overlapping morphological characters, which prevented their identification using the taxonomic keys available in the relevant literature. So, from this point on, all samples were identified by molecular analysis.

In the molecular analysis of COI, among the 135 individuals, three haplotypes were found containing two polymorphic sites and two mutations. The C+G index was 46%, the haplotype (hd) and nucleotide (π) diversities were 0.03 and 0.00005, respectively. The overall mean K2P distance for COI was 0.0000466, suggesting that samples belong to the same species. For the 16S and CR, the G+C index was 46.7% and 30%, respectively, and a single haplotype was detected in each marker.

Table 2. Maximum, minimum and mean standard length (SL), body measurements´ proportions in relation to SL, and meristic data of the "yellowish" and "gray-greenish" types, *Cichla monoculus*, *C. kelberi*, *C. piquiti* and *C. temensis*. Data for *Cichla* spp. as reported by Kullander and Ferreira (2006).

Morphometric data		**Types**		***Cichla* spp.**				**KW-H/*p***
		"Yellowish"	**"Gray-greenish"**	***C. monoculus***	***C. kelberi***	***C. temensis***	***C. piquiti***	
N (male/female)		**67 (17/50)**	**46 (19/27)**	**35**	**10**	**31**	**20**	
SL	Min.	172.0	44.3	48.1	45.5	65.2	48.1	
	Max.	315.2	343.9	275.5	375.0	298.0	275.5	
	Mean	261.3	200.5	212.0	193.1	192.6	212.0	
BD	Min.	21.1	21.2	22.0	18.2	20.9	22.0	0.6372/0.4247
	Max.	28.8	29.7	25.7	21.8	23.6	25.7	
	Mean	22.6	24.1	23.9	19.9	22.0	23.9	
CPH	Min.	10.2	9.9	11.3	8.6	10.0	11.3	0.7692/0.3824
	Max.	12.5	12.6	12.3	11.3	12.1	12.3	
	Mean	11.2	11.1	11.6	9.9	11.1	11.6	
HD	Min.	19.9	27.1	28.7	21.3	20.9	28.7	0.9851/03232
	Max.	29.6	34.6	33.7	27.3	23.6	33.7	
	Mean	29.8	30.6	32.0	24.5	22.0	32.0	
ED	Min.	6.0	6.8	7.4	6.0	6.7	7.4	1.5514/0.2157
	Max.	6.8	11.3	11.0	10.5	11.2	11.0	
	Mean	6.3	8.5	8.2	7.8	7.9	8.2	
ID	Min.	8.8	7.7	7.9	6.2	6.9	7.9	0.1619/0.6874
	Max.	9.6	9.9	9.7	8.0	8.1	9.7	
	Mean	9.2	8.8	8.9	7.0	7.5	8.9	
Meristic Data	1st DS	14-16	15-16	15-16	15-16	14-16	15-16	
	2nd DS	31-33	31-33	32-33	31-33	31-33	32-33	
	AFR	11-12	10-12	11-12	10-12	11-12	11-12	
	PFRS	13-14	13-14	13-14	14-16	14-15	13-14	
	ALL	37-51	35-51	40-48	-	42-56	40-48	
	PLL	32-38	31-39	30-38	-	32-41	30-38	
	MLL	71-81	70-79	76-83	88-109	78-104	76-83	

Abbreviations as in Table 1.

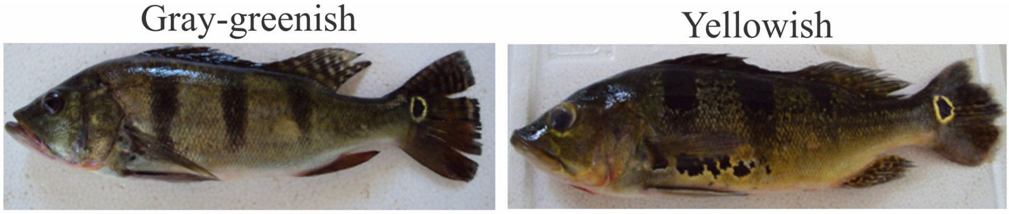

Figure 2. Gray-greenish and yellowish types of *Cichla* collected in the reservoirs of the submiddle stretch of São Francisco River.

The topologies determined by ML and BI were identical for the three markers. For COI, there was a clade formed by individuals of *Cichla* sampled from the São Francisco River and *C. kelberi* from the Tocantins River basin, and were separated from the other species with high Bayesian posterior probability (BPP = 100%) and bootstrap (94%) support values (Figure 3). For 16S, the topology also shows that specimens of *C. kelberi* from the Tocantins River form

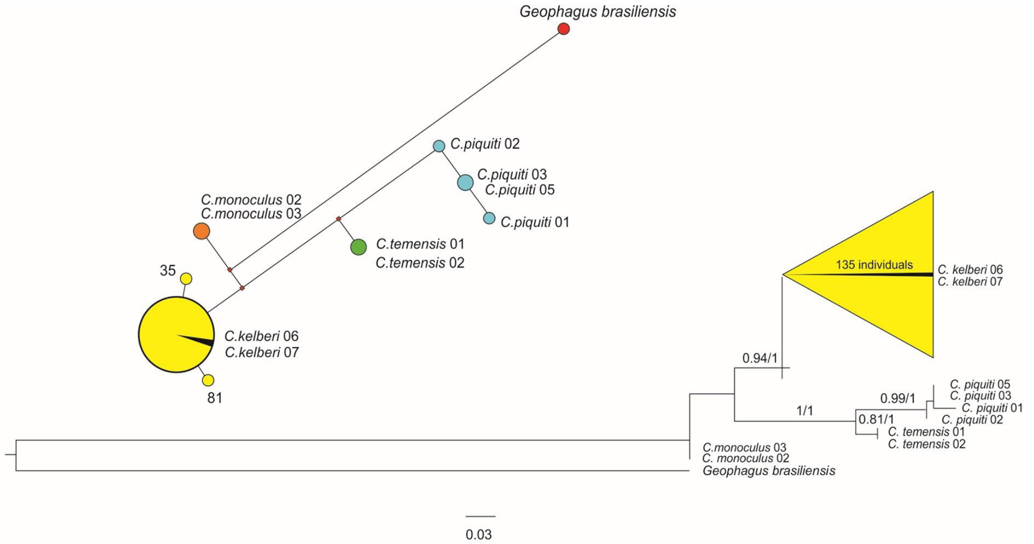

Figure 3. Bayesian consensus tree reconstructed based on sequences of *Cichla* individuals from the submiddle stretch of São Francisco River and reference sequences for COI. Haplotype network using median-joining method. Circles represent haplotypes and diameter represents frequency of haplotype.

a highly supported clade, with São Francisco River samples (BPP = 70% / bootstrap= 95%) separated from the other *Cichla* species (Supplementary material, Figure S1). Likewise, for CR, the topology consisted of a main clade made of São Francisco River samples and *C. kelberi* from Tocantins River (BPP = 99% and bootstrap = 100%) (Supplementary material, Figure S2).

4. Discussion

The sampled peacock bass and the four *Cichla* species previously reported for the São Francisco River (Pompeu and Godinho, 2003; Sato and Sampaio, 2005; Carvalho et al., 2009), exhibited considerable overlapping morphometric variation and pigmentation patterns, limiting their clear identification. However, all the 135 individuals were grouped in a well-supported clade with *C. kelberi* for the three mtDNA markers.

The dramatic lack of diversity in terms of number of haplotypes observed among the 135 individuals of *C. kelberi* for the three markers offers evidential support of a founder effect derived from a single maternal lineage. The founder effect hypothesis is reinforced by the fact that in regions of natural occurrence of *C. kelberi*, five haplotypes of CR were observed in a sample of five individuals collected in São Felix do Araguaia, while three haplotypes were detected in three individuals taken in Tucuruí Reservoir, both belonging to the Tocantins basin (Marques et al., 2016). This level of intraspecific variation of CR in the natural habitat contrasts with the single haplotype recovered in the reservoirs of the São Francisco River derived from a single invasive maternal ancestor. Our results endorse the difficulty in accrediting genetic diversity for this invasion success. Hence, in this case the colonization success may rely on other factors, such as environmental ones.

The colonization of *C. kelberi* in the reservoir cascade of the submiddle stretch of the São Francisco River may have benefited from the reduction in water turbidity (Santos et al., 2018), favouring visual predators such as the peacock bass. Espínola et al. (2010), Franco et al. (2018) and Franco et al. (2021) have highlighted that abiotic variables, such as warmer temperature, lower turbidity and higher transparency benefit the invasibility of reservoirs by *Cichla*. Moreover, the construction of the studied reservoirs (Moxotó, Delmiro Gouveia and PA IV) within a stretch of the São Francisco River, isolated by river damming, led to the local extinction of some endemic rheophilic top predators, such as the dourado *Salminus franciscanus* (Lima & Britski, 2007) and the surubim *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) (Sato and Godinho, 2003), that could have potentially preyed on juvenile forms of *Cichla*.

Control strategies could be implemented to reduce the *C. kelberi* population in the São Francisco River, such as encouragement of underwater sport fishing targeting this species, with the creation, for example, of an ecotourism plan. Moreover, the catching of this species could be opened during the closed reproduction season, when the peacock bass becomes a voracious predator of juveniles of the native threatened migratory fish species. The restocking of native top predators from the São Francisco River Basin in these reservoirs could be implemented to reduce *C. kelberi* populations. However, these activities require public policies and academic studies to support management plans that could control their population size.

The three molecular markers (COI, 16S rRNA and CR) used in this study were essential in the identification and elucidation of the two color types found in reservoirs of the São Francisco River, which were actually a single species, the *C. kelberi* (*C. ocellaris sensu lato*). This study highlighted the fact that studies focusing on species of *Cichla* should rely on a detailed molecular analysis, as past studies were apparently unable to correctly identify specimens at the species level. Other invasions of *C. kelberi*, were confirmed by molecular markers, in a lake and in reservoirs of other hydrographic basins (Marques et al., 2016; Santos et al., 2016; Diamante et al., 2017). Future study should answer the question whether this species is dominant over other congeners' species in their establishment success or if it is inherent to that genus.

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Supplementary material

Supplementary material accompanies this paper.

Figure S1. Bayesian consensus tree reconstructed based on sequences of Cichla individuals from the submiddle stretch of the São Francisco River and reference sequences for 16S rDNA. GenBank reference sequences were named as C. monoculus, C. temensis, C. kelberi and C. piquiti, followed by the last three digits of accession number.

Figure S2. Bayesian consensus tree reconstructed based on sequences of Cichla individuals from the submiddle stretch of the São Francisco River and reference sequences for Control Region. GenBank reference sequences were named as C. monoculus, C. temensis, C. kelberi and C. piquiti, followed by the last three digits of accession number.

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