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.

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...
In the context 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. intermedia, 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.
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. n° CUP 59004/00473/2013-42) were all sequenced.

Total DNA was extracted using the modified phenol-chloroform-isamyl 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 $Y_i^* = Y_i (X_i / X_i^*)$, which corrected size effects: where $Y_i^*$ = standardized morphometric measurement for each individual $i$; $Y_i$ = $i$-th morphometric measurement $Y$; $X_i$ = average standard length (SL) of all individuals; $X_i^*$ = individual standard length $i$; and $\beta$ = allometric coefficient from the linearized equation $\log Y_i = \log a + b \log X_i$, 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, FJ90812, FJ89013, 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 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, FJ90812, FJ89013, 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 deposited in GenBank under the accession numbers MW248167-MW248311 (COI), MW255385-MW255471 (16S), and MW251997-MW252032 (CR).

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 C+G index was 46.7% and 30%, respectively, and a single haplotype was detected in each marker.
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

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**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).

<table>
<thead>
<tr>
<th>Morphometric Data</th>
<th>Types</th>
<th><em>Cichla</em> spp.</th>
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<tr>
<td></td>
<td>“Yellowish”</td>
<td>“Gray-greenish”</td>
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</tr>
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<td>N (male/female)</td>
<td>67 (17/50)</td>
<td>46 (19/27)</td>
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<td>Min. 172.0</td>
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<td>Max. 315.2</td>
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<td>Min. 21.1</td>
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<td>22.0</td>
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<td></td>
<td>Max. 28.8</td>
<td>29.7</td>
<td>25.7</td>
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<tr>
<td></td>
<td>Mean 22.6</td>
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<td>23.9</td>
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<td>Mean 9.2</td>
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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.
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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.

Acknowledgements

Funding for this project was provided through the Research and Technological Development Program of the Brazilian Electricity Regulatory Agency (ANEEL 0048–044/201). CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) is acknowledged for awarding a doctoral scholarship to the first author. We thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for providing a research fellowship to Maria Raquel Moura Coimbra (grant 304518/2016-6). The authors are also indebted to Dr. Alberto Akama from the Museu Paraense Emílio Goeldi (Research Institute and Museum) – Belém (Brazil) for kindly providing muscle tissues from C. piquiti and to Dr. Mark Sabaj for providing samples of C. temensis (SUDAM – Proc. nº CUP 59004/00473/2013-42) and C. monoculus (Xingu Project).

References


Brazilian Journal of Biology, 2024, vol. 84, e248656
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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