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Genetic diversity in *Cichla piquiti* and cross-amplification for *Cichla kelberi* in the Serra da Mesa reservoir, Goiás, Brazil

Ruan Carlos Pires Faquim[1], Ramilla dos Santos Braga-Ferreira[2*], Thaís Guimarães de Castro[2], Leo Caetano Fernandes Silva[3] and Mariana Pires de Campos Telles[2,4]

[1]Universidade Estadual de Goiás, Anápolis, Goiás, Brazil. [2]Laboratório de Genética e Biodiversidade, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Av. Esperança, s/n, 74690-900, Goiânia, Goiás, Brazil. [3]Centro de Triagem de Animais Silvestres de Goiânia, Goiânia, Goiás, Brazil. [4]Escola de Ciências Médicas e da Vida, Pontifícia Universidade Católica de Goiás, Goiânia, Goiás, Brazil. *Author for correspondence. E-mail: ramillabraga@gmail.com

ABSTRACT. Species *Cichla piquiti* and *Cichla kelberi* are found in the Serra da Mesa reservoir, Goiás and are sedentary with diurnal habits. This study aimed to evaluate the magnitude and distribution of genetic variability in subpopulations of *C. piquiti* with specific microsatellite loci and to test transferability in other microsatellite markers for *C. kelberi*. We analyzed 99 individuals of *C. piquiti* from seven points to evaluate genetic diversity and structure with 10 microsatellite loci. Transferability of 75 loci was tested in *C. kelberi* to increase microsatellite markers available. Genetic structure was assessed with Bayesian clustering. Global F_{ST} for *C. piquiti* was weak (0.056), but F_{IS} (0.598) and F_{IT} (0.621) were significantly high, indicating that the mating system has a strong influence on the organization of genetic variability with most mating among related. Two genetic groups were evidenced with most individuals allocated to a single group. Transferability of microsatellite loci for *C. kelberi* had low polymorphism. The level of genetic diversity was low, increasing inbreeding and suggesting that few individuals of *C. piquiti* colonized the reservoir during its installation due founder effect. Other factors as reproductive behavior and overfishing can act to decrease genetic diversity. Therefore, we reinforce the need for genetic monitoring to avoid loss of genetic diversity that can be intensified both construction of hydropower plants and ecological and reproductive aspects in some fish species.

Keywords: bayesian analyses; genetic structure; inbreeding; microsatellite; peacock bass.

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Introduction

The richest fish biodiversity in the world is located in Brazil (estimated at 4,756) of which 3,512 are freshwater fish, representing 10% of the diversity of vertebrates in Brazil (Burgess, 2004; Froese & Pauly, 2022). However, more than 1,170 species are at risk of extinction, and of those, 26% are freshwater fish from the Actinopterygii class.

Thus, predicting and possibly mitigating the consequences of anthropogenic changes on aquatic systems requires knowledge of biological, ecological, and genetic dynamics of these species. Mainly regarding the maintenance of the diversity of the species genetic for the long-term preservation of these aquatic populations (Hutchings, 2000; Santamaria & Mendez, 2012; Herden et al., 2020).

The genus *Cichla* Block and Schneider, 1801 represents one of the main groups of piscivorous fish of the Cichlidae family in South America (Goldstein, 1973; Willis et al., 2015). These species are popularly known in Brazil as peacock basses (Tucunaré) and come from the Amazon and the Tocantins-Araguaia River basins and are widely introduced in the basins of the Paraná, Paraguai, Paraíba do Sul and Paraguaçú rivers (Gasques, Fabrin, Gonçalves, Prioli, & Prioli, 2014). Unlike most Amazonian fish, peacock basses are sedentary and diurnal, and they chase prey until successful capture. They show high dispersion across the national territory, i.e., tropical and subtropical regions, making it interesting for sport fishing (attracted by their aggressiveness), and extensive and semi-extensive fish farming (Gomeiro, Villares-Junior, & Naous, 2009). Several cases of *Cichla* species introduced in the southeastern region have been described in the literature, based on the evaluation of mtDNA regions, showing concern about the impact they may cause for the native ichthyological community (Santos, Salgueiro, Franco, Marques, & Nóbrega, 2016; Diamante et al., 2017).

Due to the aggressive and territorial behaviors of *Cichla* species, mainly in the reproductive season, natural populations of lakes and rivers may present genetic differentiation, despite connections between them

(Carvalho, Oliveira, Sampaio, & Beheregaray, 2009; Gomeiro et al., 2009). However, hitherto there is little information on the natural movement of populations of this species in rivers, lakes, and artificial dams (Hoeinghaus, Layman, Arrington, & Winemiller, 2003).

Two species of the genus *Cichla* (*C. piquiti* - blue peacock bass and *C. kelberi* - yellow peacock bass; Kullander & Ferreira, 2006) are native of the Tocantins-Araguaia River basin and are found naturally in the Serra da Mesa reservoir, Goiás, Brazil (Carvalho et al., 2009). These two species of peacock bass are targets of sport fishing since the reservoir's formation which enhanced sport fishing. Thus, it is necessary to understand that changes in the historical and contemporary landscapes of rivers and streams affect the population and demographic and genetic processes of resident fish species (Hopken, Douglas, & Douglas, 2013; Davis, Wieman, & Berendzen, 2015; Crookes & Shaw, 2016).

Understanding the mechanisms of genetic variability and intra- and interspecific maintenance of fish populations is the central theme in evolutionary and conservation genetics (Hutchings, 2000; Santamaria & Mendez, 2012). The Serra da Mesa reservoir is one of the largest reservoirs in Brazil, comprising a flooded region of the Tocantins River, where the species *C. piquiti* and *C. kelberi* appear naturally (Carvalho et al., 2009). Very few genetic studies (Trott, Callegari-Jacques, Oliveira, Langguth, & Mattevi, 2007) were made with native species in the Serra da Mesa reservoir, highlighting the necessity to verify impacts of this enterprise.

Ten microsatellite markers were developed for *C. piquiti* and transferred for species *C. kelberi* (Carvalho et al., 2009). Meanwhile, low genetic diversity was found with *C. piquiti* in different basin rivers, indicating that genetic structure and evolutionary factors can influence population dynamic of these species. Ecological and behavior aspects are affected by dams and constitute barriers to migration of fish species (Pelicice, Pompeu, & Agostinho, 2015). The impact of a big reservoir can cause, especially for small populations, the loss of genetic diversity that leads to rapid inbreeding depression, loss of heterozygosity, and consequently, of adaptability (Ellstrand & Elam, 1993; Avise, 1994). Besides, genetic data can be applied to define Evolutionarily Significant Units, seeking to propose conservationist and resilience measures (Moritz, 1994; Pelletier et al., 2020), as proposed for *Cichla temensis* Humboldt, 1821, with populations of large rivers considered to be different genetic stocks due to local adaptation (Willis et al., 2015).

Therefore, maintaining genetic variability is vital for the viability of fish recruitment programs (survival of young fish in the environment) to avoid adverse effects on ichthyofauna (Barroso, Hilsdorf, Moreira, Cabello, & Traub-Cseko, 2005; Sirol & Britto, 2006). In this context, the use of molecular markers is efficient as tools for genetic monitoring and conservation of populations (Sekino, Hara, & Taniguchi, 2002; Ortega-Villaizán, Aritaki, & Taniguchi, 2006). Microsatellite markers are useful tools to monitor genetic diversity in natural and artificial populations. Cross-amplification is a manner to obtain microsatellite loci between phylogenetically close species and allow to evaluate non-model species (Oliveira, Pádua, Zucchi, Vencovsky, & Vieira, 2006; Vieira, Santini, Diniz, & Munhoz, 2016). Unlike *C. piquiti, C. kelberi* does not have specific microsatellite markers becoming genetic transferability a manner to increase available loci.

Considering the Serra da Mesa reservoir, this enterprise has brought more urbanization, deforestation, and landscape change to the northern region of the state of Goiás (Melandri, Alencar, & Guimarães, 2015). The presence of these two species of peacock bass in the reservoir attracts sport fishing, highlighting the need for studies to assess the species behavior in an artificial environment. Therefore, this study aimed to characterize the magnitude and distribution of genetic variability, obtained with microsatellite markers, in populations of *C. piquiti* (blue peacock bass) in the Serra da Mesa reservoir, in the northern state of Goiás, Brazil. We hypothesize that the sedentary and territorial behavior of *C. piquiti* is sufficient to differentiate subpopulations throughout the Serra da Mesa reservoir. Additionally, new cross-amplification tests were carried out for *C. kelberi* to complement the study of Carvalho et al. (2009) that tested transferability for *C. kelberi* with the set of ten microsatellite makers. So, we intend to identify more microsatellite loci for this species that share geographic area with *C. piquiti*.

Material and methods

Sampling sites

This study was carried out in the Serra da Mesa reservoir, formed by the main channel of the Tocantins River and is located in the north of the state of Goiás, Brazil (13° 50' S, 48° 18' W). The reservoir comprises an area of 1,784 km², elevation of 460 m a.s.l., and was once considered the largest reservoir in Brazil in terms of

water volume, with 54.4 billion m3. The reservoir comprises mainly the municipalities of Minaçu, Campinorte, Uruaçu, Barro Alto and Niquelândia.

Sampling was performed (collection permit SISBIO - 16594) during August 2009 and December 2010, totaling 99 individuals of *C. piquiti* and 21 individuals of *C. kelberi*. The study area was divided into seven collection points (Table 1) located in four tributary rivers in the Serra da Mesa reservoir: Bagagem, Maranhão, and Traíras Rivers, in the municipalities of Niquelânida, Colinas do Sul, and Uruaçu (Figure 1). The specimen collection was performed using nets with 10 different mesh sizes: 2, 4, 3, 4, 5, 6, 7, 8, 10, 12, and 16 cm. The nets were placed both during the day and at night and reviewed every 12 hours.

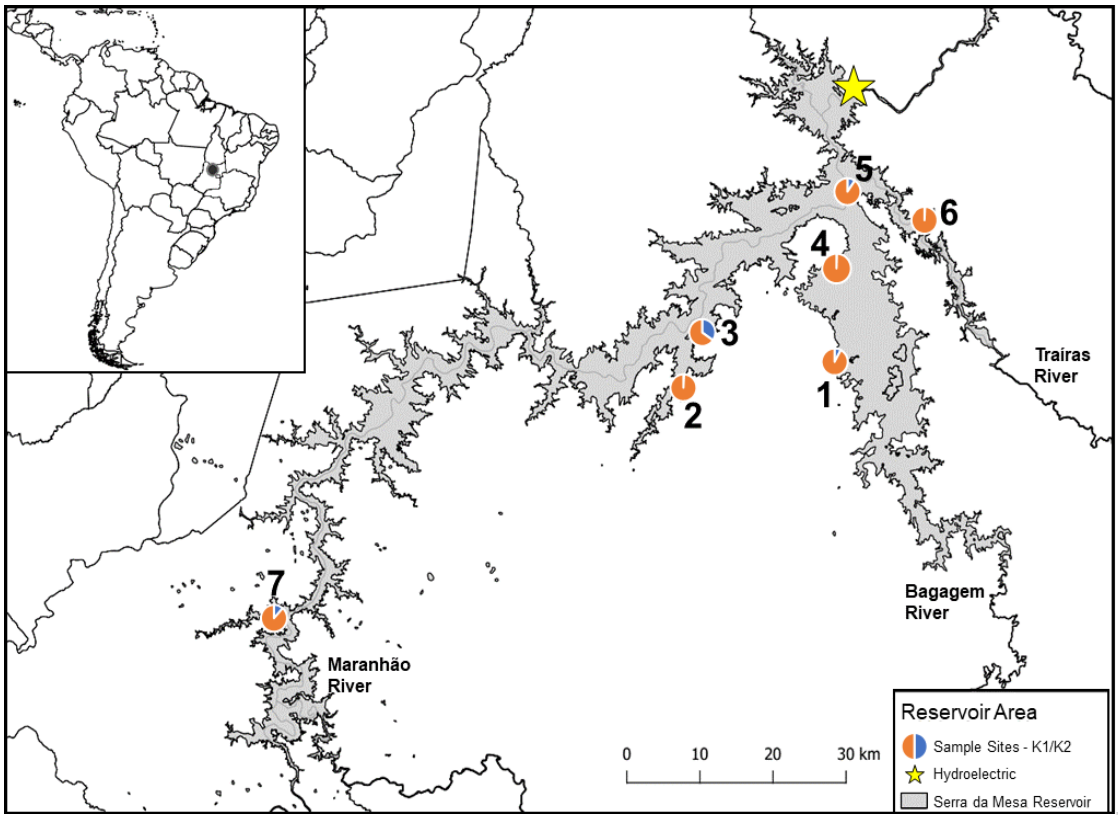

Figure 1. Sampling sites of *Cichla piquiti* and genetic clusters in Serra da Mesa reservoir, Goiás. Seven points were selected for collecting 99 individuals of this species. Genetic clusters with Bayesian analysis are visualized in each circle, in which the color represent assignment to genetic cluster (K1 is orange and K2 is blue).

Table 1. Genetic population diversity of *Cichla piquiti* at the Serra da Mesa reservoir, from six microsatellite loci (Carvalho et al., 2009). Number of individuals per population (*N*), number of alleles per population (*A*), (A_R) allelic richness, expected heterozygosity (H_E), observed heterozygosity (H_O) and fixation index (*f*).

Population	River	Longitude	Latitude	*N*	*A*	A_R	H_E	H_O	*f*
Point_1	Bagagem	-48° 19' 53.90"	-14° 09' 54.77"	16	2.3	1.8	0.149	0.083	0.439*
Point_2	Maranhão	-48° 31' 21.34"	-14° 12' 07.53"	28	1.8	1.4	0.097	0.036	0.631*
Point_3	Maranhão	-48° 29' 49.55"	-14° 07' 45.55"	10	3.0	2.8	0.465	0.200	0.570*
Point_4	Bagagem	-48° 19' 59.55"	-14° 02' 45.16"	7	1.3	1.3	0.072	0.024	0.667
Point_5	Traíras	-48° 18' 53.58"	-13° 57' 23.13"	18	2.7	2.0	0.175	0.093	0.472*
Point_6	Traíras	-48° 13' 06.40"	-13° 59' 32.80"	11	1.3	1.3	0.127	0.00	1.000*
Point_7	Maranhão	-49° 02' 44.91"	-14° 28' 25.25"	9	2.7	2.4	0.259	0.074	0.714*
Total		-	-	99	2.2	1.9	0.192	0.073	0.667

DNA extraction and amplification

Muscle tissue samples of *C. piquiti* and *C. kelberi* were collected at the bottom of the dorsal fin and kept in 96% ethanol for the conservation of the material and DNA extraction followed the protocol by Taggart, Hynes, Prodöuhl, and Ferguson (1992).

For genetic diversity of *C. piquiti*, we initially tested ten microsatellite markers developed specifically for the species, but just six loci were polymorphic for 99 individuals of *C. piquiti* (Table 2) (Carvalho et al., 2009).

The DNA of all individuals was amplified via PCR from the protocol with a final volume of 15 µL, using 5 ng of DNA template, 10X buffer (10 mM Tris-HCl, pH 8.3), 50 mM magnesium chloride (KCl), 250 µM of each dNTP, 250 µg BSA, 0.9 µM of each, and 1 U Taq DNA polymerase (Phoneutria, BR). The amplification reactions were performed in a Veriti 96-Well Thermal Cycler thermocycler (Applied Biosystems, CA). Genotyping for both species was performed using vertical electrophoresis on 6% polyacrylamide gel with a 10 bp DNA ladder marker (Invitrogen^{TM}), and the detection of amplified products using silver nitrate (Creste, Tulmann, & Figueira, 2001).

Table 2. Genetic diversity of the six microsatellite loci (Carvalho et al., 2009) analyzed in *Cichla piquiti* from the Serra da Mesa reservoir. Annealing temperature (TA), number of alleles per population (*A*), (A_R) allelic richness, expected heterozygosity (H_E), observed heterozygosity (H_O), probability of identity (*I*) and paternity exclusion (*Q*).

Loci	Sequence	Motif repeat	TA	*A*	A_R	H_E	H_O	*I*	*Q*
Tuc_03	F: TTTCATGCGGAAAAATAGCAC R: CCCTCCAGACTTCAGCTTTC	$(GT)_{21\ imperfect}$	56 ºC	4	2.1	0.208	0.000	0.666	0.099
Tuc_05	F: GCTTGTTGTGCTGGTGAGTG R: AACACTCTAAGTAGCCTTTGTTTTTG	$(CA)_9$	59 ºC	5	2.4	0.301	0.070	0.541	0.143
Tuc_09	F: TCGCTGTTGCAGCTTATCAC R: GACTGAAAGGGGGTGGAGAG	$(CA)_{10\ imperfect}$	57 ºC	3	1.9	0.186	0.205	0.671	0.088
Tuc_10	F: TCTCCCTGACCTTCAACCAG R: TGCTTAGATGAGCTGCAAGG	$(GT)_4$	61 ºC	2	1.7	0.142	0.000	0.763	0.061
Tuc_13	F: CGAAAAGACAGCATGTCAGC R: CCCATATTGACCCACTGATTC	$(CA)_{17}$	59 ºC	5	1.7	0.119	0.061	0.815	0.051
Tuc_16	F: AGATCTTCTGTGGGGAGGAG R: AAAAACAACACCATGGCAAG	$(AC)_7$	59 ºC	5	2.2	0.195	0.116	0.664	0.100
Total	-	-	-	24	2	0.192	0.075	0.010	0.436

Genetic characterization and structure

The following genetic parameters were estimated to characterize the genetic variability of *C. piquiti*: number of alleles (*A*), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, and fixation index (*f*). These genetic estimates were evaluated for each locus and per population. We performed randomization-based tests with Bonferroni correction to verify deviations in the Hardy-Weinberg equilibrium (Goudet, Raymond, Meeus, & Rousser, 1996). All of these analyses were performed using the FSTAT 2.9.3.2 software (Goudet, 2002).

Moreover, the power of individual discrimination of loci was estimated based on the probabilities of genetic identity (*I*), which is the probability of two random individuals of a population having identical genotypes (Paetkau, Calvert, Stirling, & Strobeck, 1995) and paternity exclusion (*Q*), which is the probability of excluding false paternity (Weir, 1996). These estimates were obtained for each polymorphic locus using the IDENTITY 4.0 software (Wagner & Sefc, 1999).

The magnitude and distribution of population genetic variability was evaluated just in *C. piquiti* using the F_{IT}, F_{ST}, and F_{IS} coefficients (Wright, 1951) obtained by the analysis of variance of allele frequencies (Weir & Cockerham, 1984), carried out in the FSTAT 2.9.3.2 software (Goudet et al., 1996). We used a Bayesian approach to infer existing genetic clusters, considering the genetic structure of the subpopulations sampled within the Serra da Mesa reservoir. For this, we used the STRUCTURE 2.3.4 software, assuming the assumptions of correlated allele frequencies and admixture (Pritchard, Stephens, & Donnelly, 2000). The number of *K* varied from 1 to 7, with 10,000 burn-in iterations and 100,000 steps in the Markov chain Monte Carlo (MCMC), with ten replications for each K. The choice of the best K was carried out on the STRUCTURE HARVESTER platform using the method by Evanno, Regnaut, and Goudet (2005). The coancestry coefficients for each group and individuals were calculated in the CLUMPP 1.1.2 software (Jakobsson & Rosenberg, 2007), and the cluster visualization was generated in the DISTRUCT 1.1 software (Rosenberg, 2004).

Spatial patterns of genetic variability were investigated using the simple Mantel test, correlating the geographic and genetic distance matrices. The linearized pairwise F_{ST} matrix ($F_{ST}/(1-F_{ST})$, calculated in the Arlequin 3.1 software, and the geographic distance (log) were used for this analysis (Rousset, 1997). This analysis was performed using the 'mantel' function of the 'vegan' package (Oksanen et al., 2019) in the R software (R Core Team, 2015), with 4,999 permutations to obtain the significance of the test.

Cross-amplification tests

For the cross-amplification tests with *C. kelberi* species, we used 75 microsatellite loci available in the literature to evaluate more primers of microsatellite makers. The primers were designed for the species *C. piquiti* (Carvalho et al., 2009), *Prochilodus argenteus* Spix and Agassiz, 1829 (Barbosa, Corrêa, Galzerani, Galleti, & Hatanaka, 2008), *Prochilodus costatus* Valenciennes, 1850 (Carvalho-Costa, Hatanaka, & Galetti Jr, 2006), *Piaractus mesopotamicus* Holmberg, 1887 (Calcagnotto, Russello, & Desalle, 2001), *Astyanax fasciatus* Cuvier, 1819 (Strecker, 2003), *Leporinus microcephalus* Garavello & Britski, 1988 (Morelli, Revaldaves, Oliveira, & Foresti, 2007), *Poecilia reticulata* Peters, 1859 (Paterson, Crispo, Kinnison, Hendry, & Bentzen, 2005), and *Hoplias malabaricus* Bloch, 1794 (Gondim et al., 2010). These tests were initially performed on three individuals of *C. kelberi* of different sites along the reservoir. The loci considered successfully transferred in three individuals were subsequently evaluated in a sample of 21 individuals.

The PCR protocol and verification of the amplified product used vertical electrophoresis on 6% polyacrylamide following the above conditions described for *C. piquiti*. The annealing temperatures of the 75 loci evaluated varied between 44 and 62ºC. The genetic characterization of the transferred loci occurred by analyzing the genetic parameters of expected and observed heterozygosity, and by analysis of genetic discrimination of loci.

Results

The genetic diversity found for *C. piquiti* was low because among the 10 microsatellite loci evaluated, four of them were monomorphic (Tuc04, Tuc11, Tuc12, and Tuc18). Thus, we removed these four monomorphic loci from the analysis, once they were not good evaluators of the local population genetic diversity of *C. piquiti*. The average observed and expected heterozygosity within the six remaining polymorphic loci was 0.073 and 0.192, respectively (Table 2). We found 24 alleles ranging from 1.3 to 3 in the evaluated populations (Table).

Six subpopulations had significant deviations between observed and expected heterozygosity (Table 1), leading to high inbreeding values. This occurred in the population of Point 6 that was more isolated and next to Traíras river, where *f* value was equal to 1, that is, reproduction occurs exclusively among related individuals in this population. The characterization of the loci is shown in Table 2, where the loci evaluated in the populations of the Serra da Mesa reservoir exhibit low genetic variability.

The analysis of genetic structure showed values of F_{ST} = 0.056, F_{IS} = 0.598, and F_{IT} = 0.621, all significant with $p < 0.05$. This result indicates that the reproduction mechanisms of *C. piquiti* have strong influence on the organization of genetic variation. The high F_{IT} value suggests that mating among *C. piquiti* individuals in the Serra da Mesa reservoir does not occur in a panmictic way. The F_{ST} value, despite low, was significant, suggesting a slight genetic structure among the subpopulations within the lake.

The Bayesian analysis identified two clusters with better $K = 2$ (*K1* is orange, and *K2* is blue; Figure 1. However, when observing the coancestry coefficient values (Q), we noticed that all populations are grouped in cluster 2 (blue), while the population of Point 3 obtained higher levels of admixture ($Q < 0.8$, see Table 3). The subpopulation of Point 3 obtained higher values of genetic diversity for both expected heterozygosity and allelic richness. The low genetic structure found with F_{ST} does not reflect the influence of geographic space since the Mantel test between genetic and geographical distances was not significant (-0.384; $p > 0.05$).

Table 3. Coancestry coefficient (Q) values for each cluster (*K1* and *K2*) from Bayesian analysis based on the genetic variation of *Cichla piquiti*.

Population	*K1*	*K2*	*N*
Point_1	0.079	0.921	16
Point_2	0.009	0.991	28
Point_3	0.347	0.653	10
Point_4	0.006	0.994	7
Point_5	0.096	0.904	18
Point_6	0.008	0.992	11
Point_7	0.116	0.884	9

The cross-amplification tests were not enough to identify suitable loci that permit to characterize genetic diversity of *C. kelberi*. The transferability of microsatellites for this species had low amplification success (10%) because eight of the 75 loci tested (Tuc 4, Tuc 10, Tuc 11, Tuc 16, Hmal_59, Pre 26, Ast 4, and Pcos 14;

see Table 4 and Figure 2) were transferred to *C. kelberi* genome, using 21 individuals collected in the Serra da Mesa reservoir. Of these eight loci, only the Tuc 16 was polymorphic, with three alleles in 21 individuals. The Hmal 59 locus had two alleles, yet all individuals were heterozygous for the same genotype. Consequently, we did not find microsatellite loci set suitable for population genetic analyses with *C. kelberi*.

Table 4. Genetic diversity of microsatellite loci transferred to *Cichla kelberi* from the Serra da Mesa reservoir. Annealing temperature (TA), size range (AVA), number of alleles per locus (*A*), expected heterozygosity (H_E), observed heterozygosity (H_O), probability of identity (*I*) and paternity exclusion (*Q*).

Locus	TA	AVA	*A*	H_E	H_O	*Q*	*I*
Tuc 4	54 °C	194	1.0	0.000	0.000	0.000	1.000
Tuc 10	56 °C	186	1.0	0.000	0.000	0.000	1.000
Tuc 11	56 °C	166	1.0	0.000	0.000	0.000	1.000
Tuc 16	54 °C	210-220	3.0	0.440	0.420	0.190	0.390
Par 04	50 °C	150	1.0	0.000	0.000	0.000	1.000
Hmal_59	50 °C	156-158	2.0	0.500	1.000	0.180	0.370
Pre 26	50 °C	129	1.0	0.000	0.000	0.000	1.000
Ast 4	52 °C	150	1.0	0.000	0.000	0.000	1.000
Pcos 14	46 °C	250	1.0	0.000	0.000	0.000	1.000
Geral	52 °C	-	1.2	0.470	0.710	0.040	0.860

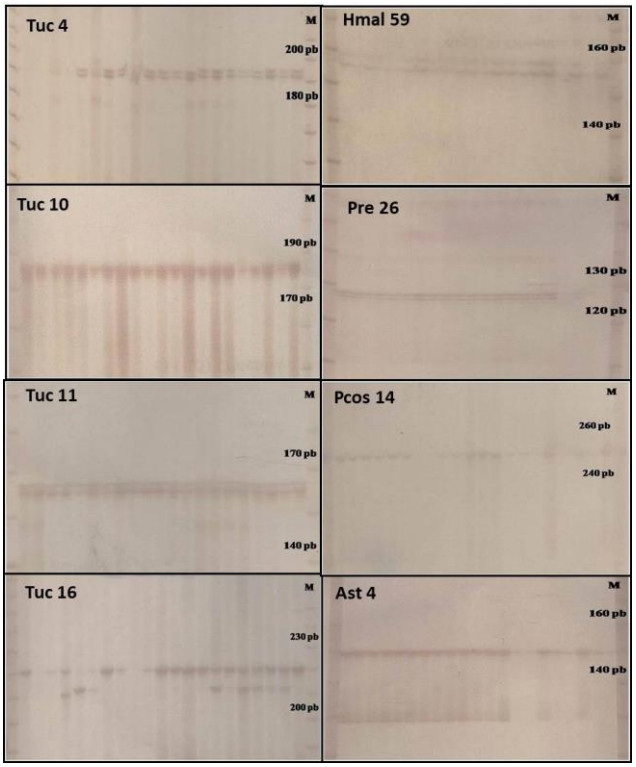

Figure 2. Polyacrylamide gel with transferred microsatellite loci in *Cichla kelberi*. Ladder 10bp is indicated in M. Just loci Tuc 16 was polymorphic while Tuc 4, Tuc 10, Tuc 11, Tuc 16, Hmal 59, Pre 26, Pcos 14 and Ast 4 were monomorphic.

Discussion

The subpopulations of *C. piquiti* from the Serra da Mesa reservoir showed low genetic variability with high intrapopulation inbreeding (F_{IS} = 0.598). The low values of expected and observed heterozygosity reflect the low genetic diversity in populations of artificial reservoirs. This is confirmed by the monomorphism in four microsatellite loci for *C. piquiti*, that the loci Tuc 4, Tuc 11 and Tuc 12 had just two alleles in populations of Amazonian basin with low observed and expected heterozygosity (Carvalho et al., 2009).

The genetic differentiation among populations was low, yet the weak genetic structure found, considering that the individuals were sampled within the same reservoir, indicates that the mating system and territorial behavior of the species can influence the organization of population genetic variability in the Serra da Mesa reservoir. As reported by Gouskov, Reyes, Wirthner-Bitterlin, & Vorburger (2016) for the species *Squalius*

cephalus Linnaeus the construction of hydroelectric plants affects the connectivity between different populations and consequently genetic structure.

Another major issue is the behavior of freshwater fish species in reservoirs, where *C. piquiti* is known to be a voracious, territorial, and sedentary predator species, which increases the mating chances among related individuals and leads to an interpopulation structuring (Gomeiro et al., 2009). However, interpopulation genetic differentiation of *C. piquiti* and *C. kelberi*, evaluated between native and introduced river populations, indicates high pairwise F_{ST} values, suggesting that different tributaries become independent genetic stocks (Carvalho, Oliveira, Sampaio, & Beheregaray, 2014). In this same study, genetic diversity was low for native and introduced populations in both species, indicating that bottleneck events act on the genetic structure of species.

The low genetic diversity of *C. piquiti* in the Serra da Mesa reservoir can be related with the colonization of the reservoir with only a few individuals of *C. piquiti*, contributing to increasing the high total inbreeding (F_{IT} = 0.621). The high inbreeding rates observed in the subpopulations of *C. piquiti* may be related to the founder effect, an ecological and genetic process resulting from the recent formation of the Serra da Mesa reservoir (< 30 years old), even though *C. piquiti* appears naturally in the Tocantins-Araguaia River basin. This process recruits few founders of a new population, causing the fixation of some alleles, and consequently, the low genetic variability caused by inbreeding (Freitas & Galetti Jr, 2005; Aho, Rönn, & Piironen, 2006), reducing the population genetic differentiation. The drastic change in local landscapes affects all types of organisms, causing the loss of countless species. The few surviving specimens will recolonize the environment, yet causing an increase in inbreeding (Aho, et al., 2006) and increases the risk of local extinction (Wright, Tregenza, & Hosken, 2008), although the specie is not classified as threatened. Founder effect in populations of reservoir reaches not just fish species but zooplankton community due to rapid growth in modified environment (Haileselasie, Mergeay, Vanoverbeke, Orsini, & De Meester, 2017).

The Bayesian analysis identified two groups (K= 2), but we emphasize that most populations are found to group 2 (in blue) and only one population had sufficient levels of admixture (Q < 0.8). This result is related to the low genetic differentiation among the populations evaluated (F_{ST} = 0.056) and confirms the existence of total inbreeding since all populations have a similar arrangement of allele frequencies. Genetic differentiation is not sufficient to separate the groups, and it is not affected by the spatial component, because there was no correlation between genetic and geographical distances.

The absence of spatial heterogeneity was a significant result because the habits of the species could influence the organization of genetic variability across geographic space. This can be justified by the collections in fairly close points (maximum distance among populations was 100 km), in which the geographical distance does not constitute a barrier to interpopulation gene flow. Moreover, intrapopulation and total inbreeding showed that the reproductive behavior of this species influences the organization of genetic variability because of the higher chance of genetic similarity over the analyzed geographical extent, due to the high degree of kinship.

The evolutionary dynamics of these subpopulations within the Serra da Mesa reservoir led to low levels of individual genetic discrimination and genetic linkage from the battery of loci in *C. piquiti*. The probability of paternity exclusion (Q) is dependent on the number and frequency distribution of alleles in the population, thus reduced levels of polymorphism or allele fixation for some markers cause low values of this parameter. In contrast, the probability of combined identity (I) must be practically zero to demonstrate that the microsatellite is an excellent marker for discriminating individuals (Collevatti, Brondani, & Grattapaglia, 1999). However, the set of loci analyzed showed high I value (0.010) and low Q value (0.436). In addition, fish dispersal strategies and life-history traits contribute to the effects of genetic variation (Pilger, Gido, Propst, Whitney, & Turner, 2017) and for *C. piquiti*, there is naturally low gene flow, due to its territorial and sedentary behavior (Gasques et al., 2014).

The species *C. kelberi* appears in the same geographic area of *C. kelberi* in the Serra da Mesa reservoir. Comparative genetic studies would be important to describe genetic diversity between two species. However, the transferability of microsatellites from different fish species to *C. kelberi* was not satisfactory because of the low success in amplifying the evaluated loci and low polymorphism. Transferability tests for *C. kelberi* were performed by Carvalho et al. (2009), but were not polymorphic in individuals of Serra da Mesa. In addition, optimized microsatellite loci of others species of fish in this study were not satisfactory to characterize the population diversity of this species in the Serra da Mesa reservoir. Even analyzing few individuals, this result demonstrates the need to develop specific microsatellite primers for *C. kelberi* since the transferred loci are not sufficient to evaluate the genetic variability of this species.

The transferability of microsatellite loci among evolutionarily close species is quite common, seeking to use universal microsatellites (Barbará et al., 2007; Vieira et al., 2016) in genetic studies of population, that have shown positive results with fish, reducing expenses related to the development of species-specific loci (Barbosa, Corrêa, Galzerani, Galleti, & Hatanaka, 2006, 2008). Positive cross-amplification among taxonomically related groups is possible due to the conservation of microsatellite flanking regions. Rico, Rico, and Hewitt (1996) had success in cross-amplification in different fish species belonging to the taxonomic groups Agnatha, Chondrichthyes, and Osteichthyes, where they found conservation of microsatellite flanking regions since the ancestral group with 470 million years. In our study, the conservation of microsatellite flanking regions may explain the transferability at a distant taxonomic level. However, the low polymorphism for the evaluated regions can be justified mainly by the analysis of individuals from the Serra da Mesa reservoir, providing low genetic diversity.

Assessing the genetic structure and the low genetic diversity in *C. piquiti* and high monomorphism in the transferred loci in *C. kelberi* highlights the existence of mechanisms leading to the homozygosity of individuals for these loci, which requires further studies since this is a native species of Serra da Mesa flooding region (Gasques et al., 2014). Besides, information regarding the genetic variability of these species, degree of inbreeding, or genetic linkage is essential for establishing management actions for fishing and even control of the populations of *C. kelberi* and *C. piquiti*. Besides, these and other fish species experience negative impacts from this enterprise such as changes from lotic to lentic environments, changes in limnological characteristics, and fragmentation of the environment, which constitutes a physical barrier to ecological and genetic processes (Oliveira, Castro, & Baptista, 2008; Almeida-Ferreira, Oliveira, Prioli, & Prioli, 2011).

Fish species encounter loss of historical genetic diversity. Thus, the formation of artificial lakes without an adequate design can aggravate this ichthyofauna peculiarity (Osborne, Perkin, Gido, & Turner, 2015). This study shows the need to evaluate the structure of fish assemblages in environments modified by hydropower plants both before and after its establishment, because they become barriers that can lead to a decrease in genetic diversity, especially for species with low movement habits (Gouskov et al., 2016).

Conclusion

Our study contributes to understanding the genetic effects on fish populations from hydropower plants, and artificial lakes. The *C. piquiti* sedentary behavior was not sufficient for genetic differentiation between populations, probably because first individuals were from same population, leading to an increase in inbreeding and low genetic differentiation. The microsatellite loci were transferred to *C. kelberi*, but there was low polymorphism for the analyzed individuals. The high rates of inbreeding observed, are probably related to the mating preference among related individuals, the founder effect and territorial behaviors, which may have been accentuated during barriers imposed by the lake formation.

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References

Aho, T., Rönn, J., & Piironen, J. (2006). Impacts of effective population size on genetic diversity in hatchery reared Brown trout (*Salmo trutta* L.) populations. *Aquaculture, 253*(1-4), 244-248. DOI: https://doi.org/10.1016/j.aquaculture.2005.09.013

Almeida-Ferreira, G. C., Oliveira, A.V., Prioli, A. J., & Prioli, S. M. A. P. (2011). Spar genetic analysis of two invasive species of *Cichla* (Tucunaré) (Perciformes: Cichlidae) in the Paraná river basin. *Acta Scientiarum. Biological Sciences, 33*(1), 9-85. DOI: https://doi.org/10.4025/actascibiolsci.v33i1.4855

Avise, J. C. (1994). *Molecular markers, natural history and evolution*. New York, NY: Chapman and Hall.

Barbará, T., Palma-Silva, C., Paggi, G. M., Bered, F., Fay, M. F., & Lexer, C. (2007). Cross-species transfer of nuclear microsatellites markers: potential and limitations. *Molecular Ecology, 16*(18), 3759-3767. DOI: https://doi.org/10.1111/j.1365-294X.2007.03439.x

Barbosa, A. C. D. R., Corrêa, T. C., Galzerani, F., Galleti, J. R. M., & Hatanaka, T. (2006). Thirteen polymorphic microsatellite loci in the Neotropical fish *Prochilodus argenteus* (Characiformes, Prochilodontidae). *Molecular Ecology Notes, 6*(3), 936-938. DOI: https://doi.org/10.1111/j.1471-8286.2006.01406.x

Barbosa, A. C. D. R., Corrêa, T. C., Galzerani, F., Galleti, J. R. M., & Hatanaka, T. (2008). Description of novel microsatellite loci in the Neotropical fish *Prochilodus argenteus* and cross-amplification in *P. costatus* and *P. lineatus*. *Genetics and Molecular Biology, 31*(1 suppl), 357-360. DOI: https://doi.org/10.1590/S1415-47572008000200032

Barroso, R. M., Hilsdorf, A. W. S., Moreira, H. L. M., Cabello, P. H., & Traub-Cseko, Y. M. (2005). Genetic diversity of wild and cultured populations of *Brycon opalinus* (Cuvier, 1819) (Characiforme, Characidae, Bryconiae) using microsatellites. *Aquaculture, 247*(1-4), 51-65. DOI: https://doi.org/10.1016/j.aquaculture.2005.02.004

Burgess, W. (2004). Checklist of the freshwater fishes of South and Central America. *Copeia, 1*(3), 714-716. DOI: https://doi.org/10.1643/OT-04-142

Calcagnotto, D., Russello, M., & Desalle, R. (2001). Isolation and characterization of microsatellite loci in *Piaractus mesopotamicus* and their applicability in other Serrasalminae fish. *Molecular Ecology Notes, 1*(4), 245-247. DOI: https://doi.org/10.1046/j.1471-8278.2001.00091.x

Carvalho, D. C., Oliveira, D. A. A., Sampaio, I., & Beheregaray, L. B. (2009). Microsatellite markers for the Amazon peacock bass (*Cichla piquiti*). *Molecular Ecology Resources, 9*(1), 239-241. DOI: https://doi.org/10.1111/j.1755-0998.2008.02425.x

Carvalho, D. C., Oliveira, D. A. A., Sampaio, I., & Beheregaray, L. B. (2014). Analysis of propagule pressure and genetic diversity in the invasibility of a freshwater apex predator: the peacock bass (genus *Cichla*). *Neotropical Ichthyology, 12*(1), 105-116. DOI: https://doi.org/10.1590/S1679-62252014000100011

Carvalho-Costa, L. F., Hatanaka, T., & Galetti Jr, P. M. (2006). Isolation and characterization of polymorphic microsatellite markers in the migratory freshwater fish *Prochilodus costatus*. *Molecular Ecology Notes, 6*(3), 818-819. DOI: https://doi.org/10.1111/j.1471-8286.2006.01356.x

Collevatti, R. G., Brondani, R. V., & Grattapaglia, D. (1999). Development and characterization of microsatellite markers for genetic analysis of a Brazilian endangered tree species *Caryocar brasiliense*. *Heredity, 83*(6), 748-756. DOI: https://doi.org/10.1046/j.1365-2540.1999.00638.x

Creste, S., Tulmann, N. A., & Figueira, A. (2001). Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Molecular Biology Reporter, 19*(4), 299-306. DOI: https://doi.org/10.1007/BF02772828

Crookes, S., & Shaw, W. P. (2016). Isolation by distance and non-identical patterns of gene flow within two river populations of the freshwater fish *Rutilus rutilus* (L.1758). *Conservation Genetics, 17*(4), 861-874. DOI: https://doi.org/10.1007/s10592-016-0828-3

Davis, D. J., Wieman, A. C., & Berendzen, P. B. (2015). The influence of historical and contemporary landscape variables on the spatial genetic structure of the rainbow darter (*Etheostoma caeruleum*) in tributaries of the upper Mississippi River. *Conservation Genetics,* 16, 167-179. DOI: https://doi.org/10.1007/s10592-014-0649-1

Diamante, N. A., Oliveira, A. V., Petry, A. C., Catelani, P. A., Pelicice, F. M., Prioli, S. M. A. P., & Prioli, A. J. (2017). Molecular analysis of invasive *Cichla* (Perciformes: Cichlidae) populations from neotropical ecosystems. *Biochemical Systematics and Ecology, 72*(1), 15-22. DOI: https://doi.org/10.1016/j.bse.2017.03.004

Ellstrand, N. C., & Elam, D. R. (1993). Population genetic consequences of small population size: Implications for Plant Conservation. *Annual Review of Ecology, Evolution, and Systematics, 24*(1), 217-242. DOI: https://doi.org/10.1146/annurev.es.24.110193.001245

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology, 14*(8), 2611-2620. DOI: https://doi.org/10.1111/j.1365-294X.2005.02553.x

Freitas, P. D., & Galetti, J. R. P. M. (2005). Assessment of the genetic diversity in five generations of commercial broodstock line of *Litopenaeus vannamei* shrimp. *African Journal of Biotechnology, 4*(12), 1362-1367. DOI: https://doi.org/10.5897/AJB2005.000-3283

Froese, R., & Pauly, D. (2022). *FishBase*. Retrieved from www.fishbase.org, version.

Gasques, L. S., Fabrin, T. M. C., Gonçalves, D. D., Prioli, S. M. A. P., & Prioli, A. J. (2014). A introdução do gênero *Cichla* [Block e Schneider, 1801] na planície de inundação do Alto Rio Paraná. *Arquivos de Ciências Veterinárias e Zoologia, 17*(4), 261-266. DOI: https://doi.org/10.25110/arqvet.v17i4.2014.5027

Goldstein, R. J. (1973). *Cichlids of the world*. Neptune Township, NJ: T.F.H. Publication.

Gomeiro, L. M., Villares-Junior, G. A., & Naous, F. (2009). Pesca experimental do tucunaré *Cichla kelberi* Kullander introduzido em um lago artificial no sudeste brasileiro. *Revista Brasileira de Engenharia de Pesca, 4*(2), 11-19. DOI: https://doi.org/10.18817/repesca.v4i2.156

Gondim, S. G. C. A., Rezende, L. V., Brondani, R. P. V., Collevatti, R. G., Silva-Júnior, N. J., Pereira, R. R., & Telles, M. P. C. (2010). Development of microsatellite markers for *Hoplias malabaricus* (Erythrinidae). *Genetics and Molecular Research, 9*(3), 1513-1517. DOI: https://doi.org/10.4238/vol9-3gmr877

Goudet, J. (2002). *FSTAT a program to estimate and test gene diversities and fixation indices: Version 2.9.3.2*. Retrieved from https://www2.unil.ch/popgen/softwares/fstat.htm

Goudet, J., Raymond, M., Meeus, T., & Rousser, F. (1996) Testing differentiation in diploid population. *Genetics*, 144(4), 1933-1940. DOI: https://doi.org/10.1093/genetics/144.4.1933

Gouskov, A., Reyes, M., Wirthner-Bitterlin, L., & Vorburger, C. (2016). Fish population genetic structure shaped by hydroelectric power plants in the upper Rhine catchment. *Evolutionary Applications, 9*(2), 394-408. DOI: https://doi.org/10.1111/eva.12339

Haileselasie, T., H., Mergeay, J., Vanoverbeke, J., Orsini, L., & De Meester, L. (2017). Founder effects determine the genetic structure of the water flea *Daphnia* in Ethiopian reservoirs. *Limnology and Oceanography, 63*(2), 915-926. DOI: https://doi.org/10.1002/lno.10678

Herden, T., Bönisch, M., & Friesen, N. (2020). Genetic diversity of *Helosciadium repens* (Jacq.) WDJ Koch (Apiaceae) in Germany, a Crop Wild Relative of celery. *Ecology and Evolution, 10*(2), 875-890. DOI: https://doi.org/10.1002/ece3.5947

Hoeinghaus, D. J., Layman, C. A., Arrington, D. A., & Winemiller, K. O. (2003). Movement of *Cichla* (Cichidae) in Venezuelan flooplaain river. *Neotropical Ichthyology, 1*(2), 121-126. DOI: https://doi.org/10.1590/S1679-62252003000200006

Hopken, M. W., Douglas, M. R., & Douglas, M. E. (2013). Stream hierarchy defines riverscape genetics of a North American desert fish. *Molecular Ecology, 22*(4), 956-971. DOI: https://doi.org/10.1111/mec.12156

Hutchings, J. A. (2000). Collapse and recovery of marine fishes. *Nature, 406*(6798), 882-885. DOI: https://doi.org/10.1038/35022565

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics, 23*(14), 1801-1806. DOI: https://doi.org/10.1093/bioinformatics/btm233

Kullander, S., & Ferreira, E. (2006). A review of the South American cichlid genus *Cichla*, with descriptions of nine new species (Teleostei: Cichlidae). *Ichthyological Exploration of Freshwaters, 17*(4), 289-398.

Melandri, V., Alencar, J., & Guimarães, A. E. (2015). The influence of the area of the Serra da Mesa Hydroelectric Plant, State of Goiás, on the frequency and diversity of anophelines (Diptera: Culicidae): a study on the effect of a reservoir. *Revista da Sociedade Brasileira de Medicina Tropical, 48*(1), 33-38. DOI: https://doi.org/10.1590/0037-8682-0225-2014

Morelli, K. A., Revaldaves, E., Oliveira, C., & Foresti, F. (2007). Isolation and characterization of eight microsatellite loci in *Leporinus macrocephalus* (Characiformes: Anostomidae) and cross-species amplification. *Molecular Ecology Notes, 7*(1), 32-34. DOI: https://doi.org/10.1111/j.1471-8286.2006.01484.x

Moritz, C. (1994). Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution, 9*(10), 373-375. DOI: https://doi.org/10.1016/0169-5347(94)90057-4

Oksanen, J., Blanchet, F. F., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., ... & Wagner, H. (2019). Vegan: mantel and partial mantel tests for dissimilarity matrices. *R package version, 2, 5-3*.

Oliveira, E. J., Pádua, J. G., Zucchi, M. I., Vencovsky, R., & Vieira, M. L. C. (2006). Origin, evolution and genome distribution of microsatellites. *Genetics Molecular Biology, 29*(2), 294-307. DOI: https://doi.org/10.1590/S1415-47572006000200018

Oliveira, R. B. S., Castro, C. M., & Baptista, D. F. (2008). Desenvolvimento de índices multimétricos para utilização em programas de monitoramento biológico da integridade de ecossistemas aquáticos. *Oecologia Brasiliensis, 12*(3), 487-505. DOI: https://doi.org/10.4257/oeco.2008.1203.09

Ortega-Villaizán, M. M. R., Aritaki, M., & Taniguchi, N. (2006). Pedigree analysis of recaptured fish in the stock enhancement program of spotted halibut *Verasper variegates*. *Fisheries Science, 72*(1), 48-52. DOI: https://doi.org/10.1111/j.1444-2906.2006.01115.x

Osborne, M. J., Perkin, S. J., Gido, K. B., & Turner, T. F. (2015). Comparative riverscape genetics reveals reservoirs of genetic diversity for conservation and restoration of Great Plains fishes. *Molecular Ecology, 23*(23), 5663-5679. DOI: https://doi.org/10.1111/mec.12970

Paterson, I. G., Crispo, E., Kinnison, M. T., Hendry, A. P., & Bentzen, P. (2005). Characterization of tetranucleotide microsatellite markers in guppy (*Poecilia reticulata*). *Molecular Ecology Notes, 5*(2), 269-271. DOI: https://doi.org/10.1111/j.1471-8286.2005.00895.x

Paetkau, D., Calvert, W., Stirling, I., & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology Resources, 4*(3), 347-354. DOI: https://doi.org/10.1111/j.1365-294x.1995.tb00227.x

Pelicice, F. M., Pompeu, P. S., & Agostinho, A. A. (2015) Large reservoirs as ecological barriers to downstream movements of Neotropical migratory fish. *Fish and Fisheries, 16*(4), 697-715. DOI: https://doi.org/10.1111/faf.12089

Pelletier, M. C., Ebersole, J., Mulvaney, K., Rashleigh, B., Gutierrez, M. N., Chintala, M., ... & Lane, C. (2020). Resilience of aquatic systems: review and management implications. *Aquatic Sciences,* 82(2), 1-41. DOI: 10.1007/s00027-020-00717-z

Pilger, T. J., Gido, K. B., Propst, D. L., Whitney, J. E., & Turner, T. F. (2017). River network architecture, genetic effective size, and distributional patterns predict differences in genetic structure across species in a dryland stream fish community. *Molecular Ecology, 26*(10), 2687-2697. DOI: https://doi.org/10.1111/mec.14079

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics,* 155(2), 945-959. DOI: https://doi.org/10.1093/genetics/155.2.945

R Core Team. (2015). *R: A language and environment for statistical computing*. Vienna, AT: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Rico, C., Rico, I., & Hewitt, G. (1996). 470 million years of conservation of microsatellite loci among fish species. *Proceeding of Royal Society of London in Biology Sciences, 263*(1370), 549-557. DOI: https://doi.org/10.1098/rspb.1996.0083

Rosenberg, N. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes, 4*(1), 137-138. DOI: https://doi.org/10.1046/j.1471-8286.2003.00566.x

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics, 145*(4), 1219-1228. DOI: https://doi.org/10.1093/genetics/145.4.1219

Santamaria, L., & Mendez, P. F. (2012). Evolution in biodiversity policy–current gaps and future needs. *Evolutionary Applications, 5*(2), 202-218. DOI: https://doi.org/10.1111/j.1752-4571.2011.00229.x

Santos, L. N., Salgueiro, F., Franco, A. C. S., Marques, A. C. P. B., & Nóbrega, F. (2016). First record of the invasive blue peacock cichlid *Cichla piquiti* Kullander and Ferreira 2006 (Cichliformes: Cichlidae) in the Paraíba do Sul river basin, south eastern Brazil. *BioInvasions Records, 5*(4), 267-275. DOI: http://dx.doi.org/10.3391/bir.2016.5.4.12

Sekino, M., Hara, M., & Taniguchi, N. (2002). Loss of microsatellite and mitochondrial DNA variation in hatchery strains of Japanese flounder *Paralichthys olivaceus*. *Aquaculture, 213*(1-4), 101-122. DOI: https://doi.org/10.1016/S0044-8486(01)00885-7

Sirol, R. N., & Britto, S. G. (2006). Conservação e manejo da ictiofauna: repovoamento. In M.G. Nogueira, R. Henry,. & A. Jorcin (Eds.), *Ecologia de reservatórios: impactos potenciais, ações de manejo e sistemas em cascatas* (p. 275-284). São Carlos, SP: RiMA.

Strecker, U. (2003). Polymorphic microsatellites isolated from the cave fish *Astyanax fasciatus*. *Molecular Ecology Notes, 3*(1), 150-151. DOI: https://doi.org/10.1046/j.1471-8286.2003.00386.x

Taggart, J. B., Hynes, R. A., Prodöuhl, P. A., & Ferguson, A. (1992). A simplified protocol for routine total DNA isolation from salmonid fishes. *Journal of Fish Biology, 40*(1), 963-965. DOI: 10.1111/j.1095-8649.1992.tb02641.x

Trott, A., Callegari-Jacques, S. M., Oliveira, L. F. B., Langguth, A., & Mattevi, M. S. (2007). Genetic diversity and relatedness within and between species of the genus *Oligoryzomys* (Rodentia; Sigmodontinae). *Brazilian Journal Biology, 67*(1): 153-160. DOI: https://doi.org/10.1590/S1519-69842007000100021

Vieira, M. L. C., Santini, L., Diniz, A. L., & Munhoz, C. F. (2016). Microsatellite markers: what they mean and why they are so useful. *Genetics Molecular Biology, 39*(3), 312-328. DOI: https://doi.org/10.1590/1678-4685-GMB-2016-0027

Wagner, H. W., & Sefc, K. M. (1999). *Identity 1.0.* Vienna: AT: University of Agricultural Sciences.

Weir, B. S. (1996). *Genetic data analysis II: methods for discrete population genetic data*. Sunderland, MA: Sinauer Associates Inc.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution Resources, 38*(6), 1358-1370. DOI: https://doi.org/10.2307/2408641

Willis, S. C., Winemiller, K. O., Montana, C. G., Macrander, J., Reiss, P., Farias, I. P., & Ortí, G. (2015). Population genetics of the speckled peacock bass (*Cichla temensis*), South America's most important inland sport fishery. *Conservation genetics, 16*(6), 1345-1357. DOI: https://doi.org/10.1007/s10592-015-0744-y

Wright, S. (1951). The genetical structure of populations. *Annual Eugenics, 15*(1), 323-354. DOI: https://doi.org/10.1111/j.1469-1809.1949.tb02451.x

Wright, L. I., Tregenza, T., & Hosken, D. J. (2008). Inbreeding, inbreeding depression and extinction. *Conservation Genetics,* 9(1), 833-843. DOI: https://doi.org/10.1007/s10592-007-9405-0