Acta Scientiarum. Biological Sciences
ISSN: 1679-9283
eduem@uem.br
Universidade Estadual de Maringá
Brasil

Briñez, Boris; Júlio Júnior, Horacio Ferreira; Prioli, Sônia Maria Alves Pinto; Maniglia, Thiago Cintra; Prioli, Alberto José
Molecular identification of Cichla (Perciformes: Cichlidae) introduced in reservoirs in Southern Brazil
Acta Scientiarum. Biological Sciences, vol. 35, núm. 2, abril-junio, 2013, pp. 233-239
Universidade Estadual de Maringá
Maringá, Brasil

Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i2.10684

Molecular identification of *Cichla* (Perciformes: Cichlidae) introduced in reservoirs in Southern Brazil

Boris Briñez[1*], Horacio Ferreira Júlio Júnior[2], Sônia Maria Alves Pinto Prioli[3], Thiago Cintra Maniglia[2] and Alberto José Prioli[2]

*[1]Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, Paraná, Brazil. [2]Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [3]Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: borisbrinez@hotmail.com*

ABSTRACT. Species of peacock bass were introduced in several watersheds in South America and worldwide, mainly due to its importance to sport fishing, by being a fighting fish. A recent revision of the genus *Cichla* showed that the species introduced in reservoirs of the South, Southeast and Northeast regions of Brazil are two new species, described as *Cichla kelberi* (yellow peacock bass) and *Cichla piquiti* (blue peacock bass), erroneously identified as *C. monoculus* and *C. ocellaris*. With the purpose to identify the populations of *Cichla* in Paranapanema and Paraná rivers, a total of 323 base pairs (bp) of the mtDNA control region were sequenced, obtained from 84 specimens of *Cichla* in six different localities (Tapajós river, Solimões river, Capivara, Taquaruçu and Rosana reservoirs in the Paranapanema river, and in the upper Paraná river floodplain). The analyses revealed the genetic diversity of *Cichla monoculus*, introduced into the Capivara reservoir, originally from the region of Manaus (Amazonas State), and spread in the reservoirs downstream (Taquaruçu and Rosana). The occurrence of the same haplotypes in the three reservoirs suggests one single introduction. This study confirmed the introduction of *Cichla* in the Capivara reservoir and showed the genetic diversity of *Cichla* in the Paranapanema river.

Keywords: species introduction, *C. monoculus*, mitochondrial DNA, peacock bass, Paraná river.

Identificação molecular de *Cichla* (Perciformes: Cichlidae) introduzidas nos reservatórios do Sul do Brasil

RESUMO. Espécies de tucunaré foram introduzidas em inúmeras bacias hidrográficas da América do Sul e em outras regiões do planeta, principalmente pelas suas características esportivas, de peixe lutador. Revisão recente das espécies do gênero *Cichla* mostraram que as espécies que foram introduzidas nos reservatórios das regiões Sul, Sudeste e Nordeste, são duas espécies novas, descritas como *Cichla kelberi* (tucunaré amarelo) e *Cichla piquiti* (tucunaré azul) identificadas erroneamente como *C. monoculus* e *C. ocellaris*. Com o objetivo de identificar as populações de *Cichla* presentes no rio Paranapanema e Paraná, foram sequenciadas um total de 323 pares de bases (pb) da região controle (mtDNA) obtidas de 84 espécime de *Cichla* em seis localidades diferentes (rio Tapajós, rio Solimões, Reservatórios de Capivara, Taquaruçu, Rosana localizados no rio Paranapanema e na bacia do alto rio Paraná. Os dendrogramas e as análises das populações revelaram fortes evidências de que *Cichla monoculus* foi introduzida no reservatório de Capivara, proveniente da região de Manaus e se dispersou para os reservatórios localizados a jusante (Taquaruçu e Rosana). A ocorrência dos mesmos haplótipos nos três reservatórios sugerem uma única introdução. Este trabalho confirma a introdução de *Cichla* no reservatório de Capivara e revela a diversidade genética das espécies presentes no rio Paranapanema.

Palavras-chave: espécie introduzida, *C. monoculus*, DNA mitocondrial, Tucunaré, rio Paraná.

Introduction

The introduction of invasive species is the second leading cause of extinction of both animals and plants, being surpassed only by habitat changes, such as the construction of large dams and deforestation. Species of the genus *Cichla* (peacock bass) are restricted to the Neotropical region, and are the representatives of the family Cichlidae that reach the largest size, have considerable economic importance in the Amazon region, both in commercial and recreational fishing. Recently, Kullander and Ferreira (2006) reviewed the species of the genus *Cichla* and observed that the species introduced in the reservoirs of the South, Southeast and Northeast regions are two new species, described as *Cichla kelberi* (yellow peacock bass) and *Cichla piquiti* (blue peacock bass), mistakenly

identified as *C. monoculus* and *C. ocellaris*. Afterwards Oliveira et al. (2008) reported the presence of *C. monoculus* in the Capivara reservoir and more recently Carvalho et al. (2009) showed the genetic origin of the invasive peacock bass populations in four major river basins of Minas Gerais State.

The species of peacock bass, mainly due to their sporting characteristics, have been introduced in many river basins of South America and worldwide. In the upper Paraná river floodplain, species of *Cichla*, such as *C. kelberi* (yellow peacock bass) and *C. piquiti* (blue peacock bass) were introduced in reservoirs of the rivers Grande, Tietê and Paranaíba. The capture of large numbers of immature specimens suggests a high reproductive success in these environments (ESPÍNOLA et al., 2010). In general, it is possible to find these two species and hybrids in the floodplain (OLIVEIRA et al., 2006). Dams constructed before the introduction of the species in the Paraná river prevented their spread to the Paranapanema river. As shown by Oliveira et al. (2006, 2008), the population of the Capivara reservoir is characterized as *Cichla monoculus* from an independent introduction. The present study aimed to identify *Cichla* species introduced in the reservoirs of the Paranapanema river and upper Paraná river Basin.

Material and methods

Samples collection

This study encompassed three reservoirs of the Paranapanema river (Rosana, Taquaruçu and Capivara), arranged in sequence along the river; the Paraná river in the region of Porto Rico; and samples of the rivers Solimões and Tapajós (Table 1, Figure 1). Muscle tissue samples from eighty-four specimens of *Cichla* (peacock bass) were collected between March and December 2008, and preserved in absolute ethanol and kept at -20°C. The adult specimens of peacock bass were fixed in 40% formaldehyde and deposited in the Ichthyology Collection of Nupélia (Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura), Cod: Nupélia 6838.

Extraction and Amplification of DNA

DNA was extracted according to Monesi et al. (1998) with some modifications (PRIOLI et al., 2002). The amplification of mtDNA fragments by PCR was based on Prioli et al. (2002). The fragment was amplified by the pair of primers L A21 mtDNA control region (5'-AGAGCGTCGGTCTTGT AAACC-3') (CRONIN et al., 1993) and H16498 (5'-CCTGAAGTAGGAACCAGATG-3') (MEYER et al., 1990), containing approximately 550 base pairs (bp). This is constituted of the sequence of the gene $tRNA^{Pro}$, which encodes the tRNA of the amino acid proline, and the rest of the fragment contains the hyper variable sequence 5' of the control region of the mitochondrial DNA molecule.

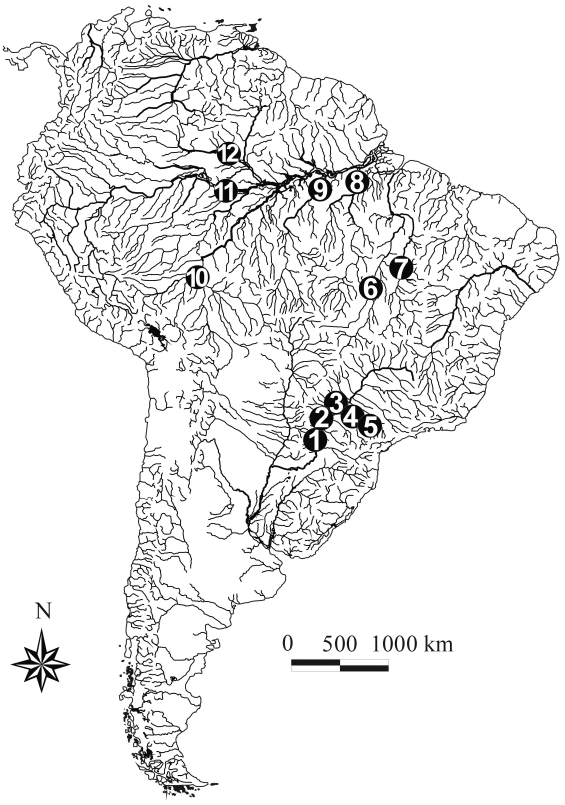

Figure 1. Map of South America showing the study regions. Locality 1: Itaipu reservoir in the Paraná river (24°05' S; 25°33' W), 2: Upper Paraná river floodplain near Porto Rico town (22°78' S; 53°31' W), 3: Rosana reservoir in the Paranapanema river (22°60' S; 52°86' W), 4: Taquaruçu reservoir in the Paranapanema river (22°54' S; 52°00' W), 5: Capivara reservoir in the Paranapanema river (22°66' S; 51°33'W), 6: Araguaia river (11°37' S; 50°39' W), 7: Tocantins river (9°75' S; 48°36' W), 8: Xingu river (2°29' S; 52°01' W), 9: Tapajós river (2°41' S; 54°71' W), 10: Madeira river (9°52' S; 65°18' W), 11: Solimões river (4°04' S; 63°09' W), 12: Negro river (0°58' S; 62°54' W).

The PCR reaction volume was 25 μL, containing Tris-KCl (20 mM Tris-HCl pH 8.4 with 50 mM KCl), 1.5 mM $MgCl_2$, 2.5 μM of each *primer*, 0.1 mM of each dNTP, 2.5 U of *Taq* DNA polymerase, 15 ng of DNA and Milli-Q water to complete the 25 μL volume. The *mixture* was placed in a thermocycler PTC-200 (MJ Research) and DNA denatured at 94°C for 4 minutes. The amplification occurred during 40 cycles, with each one consisting of denaturation at 94°C for 15 seconds, annealing at 59°C for 30 seconds and 72°C for 2 minutes, followed by a final extension of 72°C for 10 minutes. The DNA was amplified and quantified, and in order to eliminate the

excess of primers and dNTPs that could interfere with the sequencing, a purification process was performed following the protocol of Rosenthal et al. (1993).

Sequencing

The obtained fragments were sequenced according to the manufacturer's instructions using the primer H16498. About 50 ng of DNA of the final product of each PCR was used as sample for the sequencing in the automatic sequencer MegaBace (Amersham) following the manufacturer's instructions.

The sequences were thus aligned using the program CLUSTAL W (THOMPSON et al., 1994) and manually edited with the program BIOEDIT (HALL, 1999). The *p*-distances and the numbers of polymorphic nucleotides were calculated using MEGA 4.0 (TAMURA et al., 2007).

The evolutionary model was selected using the programs PAUP 4.0 (SWOFFORD, 2002) and MODELTEST 3.7 (POSADA; CRANDALL, 1998). The corrected Akaike Information Criterion (AlCc) and Bayesian Information Criterion (BIC) were used to select the nucleotide substitution model. To construct the phylogenetic tree, we used the maximum likelihood method on the selected model, and bootstrap based on 10,000 resamplings. The posterior distribution of trees was approximated by using the Markov chain Monte Carlo as implemented in MrBayes (HUELSENBECK et al., 2001). The sequences of GenBank DQ841871 and DQ841865 were used as external groups for *C. ocellaris* and *C. orinocensis*, respectively. The haplotype diversity (*h*) and nucleotide diversity (π) were determined using the programs Arlequin 3.1 (EXCOFFIER et al., 2007) and DNAsp (LIBRADO; ROZAS, 2009).

GenBank Sequences

Besides the sequences of the mtDNA control region of *Cichla* obtained from the specimens collected in the present study, ninety-seven sequences of the GenBank were used: (AY836726-32, AY836748-50) reported by Oliveira et al. (2006), (DQ841872-99) reported by Willis et al. (2007), and (DQ778666-712) reported by Renno et al. (2006).

Results

Sequences with a total of 323 base pairs (bp) of the mtDNA control region were obtained from eighty-four specimens of *Cichla* (GenBank accession numbers FJ872833 to FJ 872916) in six different locations (reservoirs Capivara, Taquaruçu and Rosana in the Paranapanema river, and rivers Tapajós, Solimões and Paraná in the region of Porto Rico) (Table 1). Forty-nine haplotypes were found in the samples of *C. monoculus* collected in Amazonas and four haplotypes were found in the samples collected in the Paranapanema river (Capivara, Taquaruçu and Rosana reservoirs) and Paraná river in the Porto Rico region. In the Paraná State, four haplotypes of *C. kelberi* were also found (region of Porto Rico and Taquaruçu reservoir).

Table 1. Location and number of specimens of two species of *Cichla* examined in the present study.

Basin	Locality	*C. monoculus*	*C. kelberi*	Total
Paranapanema*	Rosana	21		21
Paranapanema*	Taquaruçu	17	1	18
Paranapanema*	Capivara	17		17
Paranapanema**	Capivara	7		7
Paraná*	Porto Rico	2	22	24
Paraná**	Porto Rico		5	5
Amazônica*	Solimões; Tapajós	4		4
Amazônica/Orinoco**	Solimões; Tapajós; Xingu; Madeira; Negro; Orinoco	79	—	79
Tocantins/Araguaia**	Porto Nacional; Ipueiras; Pedro Afonso; São Felix de Araguaia	—	10	10
Total		147	38	185

*Specimens collected for this study; **Sequences available in GenBank.

The number of mutations and haplotypes, and haplotype and nucleotide diversity presented in the studied Basins, are listed in the Table 2.

Table 2. Haplotype and nucleotide diversity of the populations of *C. monoculus*.

Basin	N	S	Hap	*h*	π
Amazônica/Orinoco-Paraná/Paranapanema	152	119	52	0.888	0.06612
Amazônica/Orinoco	83	118	49	0.966	0.07865
Paraná/Paranapanema	69	13	4	0.532	0.01867

N=Samples; s=Number of mutations; Hap=Number of haplotypes; h=Haplotype diversity; π=Nucleotide diversity.

Of the four haplotypes of *C. monoculus* observed in the Upper Paraná basin, the first haplotype was found in the reservoirs Capivara, Taquaruçu and Rosana, and also in samples of the Solimões river. But it was not observed in the samples of Porto Rico. The haplotype frequency was 31%. The second haplotype was found in all sampling sites (Capivara, Rosana, Taquaruçu and Porto Rico). Importantly, this haplotype is 99% similar to the haplotype of the Tapajós river collected in the region of Santarém, with a frequency of 64%. The third haplotype was found in Capivara, Taquaruçu and Porto Rico, and differs from the others by the deletion of two thymines, with a frequency of 2.4%. The fourth haplotype was found only in Porto Rico, with a single thymine nucleotide deletion compared to the second haplotype. The haplotype frequency was 2.4% (Table 3).

Table 3. Nucleotide polymorphisms in the hypervariable sequence (~323 bp) of the mtDNA control region from *Cichla* invasive and native populations. The samples are indicated by the number identification: 2. Upper Paraná river floodplain; 3.Rosana reservoir; 4. Taquaruçu reservoir; 5. Capivara reservoir; 9. Tapajós river; 11. Solimões river. Specimen: Cichla identification. Haplotypes: Hapl-Cmn. *C. monoculus*; Hapl-Kbr. *C. kelberi*. Entire sequences at Genbank: FJ872833 to FJ872916.

Sampling locations	Specimen	Haplotypes	Identification	00000000000011111111111111111223 01455677779902234445556777783 60 22334534783904634794890035708 73
11	TUC-121	Hapl-Cmn1	*C. monoculus*	TGTATCCAGTCTCCACCAACATCTGTTCATC
1	TUC-122	Hapl-Cmn2	*C. monoculus*	......T.........G..........
3-4-5-11	TUC-123-143	Hapl-Cmn3	*C. monoculus*	......T..........................
2-3-4-5	TUC-144-164	Hapl-Cmn4	*C. monoculus*	C.C.CATCA...TT..-....CTC......T
9	TUC-165	Hapl-Cmn5	*C. monoculus*	C.C.CATCA.T.TT..-....CTC......T
2-4-5	TUC-166-181	Hapl-Cmn6	*C. monoculus*	C.C.CATCA...TT..-....CTC......
2	TUC-182	Hapl-Cmn7	*C. monoculus*	C.CCAATCA...TT..-....CTC......T
2	TUC-183-185	Hapl-Kbr1	*C. kelberi*	CA....TT.A.CTTGT.TT-..T.ACATGCT
4	TUC-186	Hapl-Kbr2	*C. kelberi*	CA....TT.A.CTTGT.TT-..T.ACAT.CT
2	TUC-187	Hapl-Kbr3	*C. kelberi*	CA....TT.A..TTGT.TT-..T.ACATGCT
2	TUC-188-204	Hapl-Kbr4	*C. kelberi*	CA....TT.A..TTGT.TT-G.T.ACAT.CT

The haplotype diversity and the nucleotide diversity were, as expected, higher in samples from the Amazonas basin than in the upper Paraná basin. The genetic diversity of the samples of each location is presented in Table 2. These parameters were not calculated for *C. kelberi* owing the bilateral hybridization between the two congeners introduced in this basin (GRAÇA; PAVANELLI, 2007; OLIVEIRA et al., 2006).

The corrected Akaike Information Criterion (AICC) and Bayesian Information Criterion (BIC) of ModelTest have indicated the HKY + G as the optimal evolutionary model for the *D-loop* sequences. The frequencies of the nucleotide bases were freqA = 0.4207, freqC = 0.1692, freqG = 0.0952, freqT = 0.3148 and of the replacing models Ti / Tv ratio = 5.0888; the parameter gamma distribution (G) was G = 0.3370 and the proportion of invariable sites (I) was I = 0. The transition/transversion ratios were K1 = 4.647 (purines) and K2 = 9.613 (pyrimidines) and the general transition/transversion ratio R= [A*G*k1 + T*C*k2]/[(A+G)*(T+C)] was R = 1.976.

The heuristic inference (maximum likelihood) (Figure 2) generated by the PAUP, resulted in a tree with six major clades denominated groups. The topology was not affected by the inclusion of the two external groups. The phylogenetic reconstruction placed some species available in GenBank (AY836739, AY836717, AY836718, AY836719, AY836742, AY836745) as *C.* cf. *monoculus* being *C. kelberi*. This is because the description of the latter species was made subsequently (KULLANDER; FERREIRA, 2006). The tree shows six main clades. The first clade contains *C. kelberi* from Araguaia (S. Willis, personal communication), Itaipu, Tocantins and the upper Paraná river basin in the region of Porto Rico (present study). The second clade contains *C. monoculus* from the reservoirs Taquaruçu, Rosana, Capivara and the upper Paraná river basin in the region of Porto Rico, Tapajós river in the region of Santarém (present study), and the rivers Tapajós, Amazonas and Xingu (WILLIS et al., 2007). The third clade contains *C. monoculus* from the Solimões river, reservoirs Taquaruçu, Capivara and Rosana (present study) and the rivers Solimões, Xingu and Tapajós reported by Willis et al. (2007) and Renno et al. (2006). The fourth clade includes *C. monoculus* from the rivers Madeira, Negro and Orinoco reported by Willis et al. (2007). The fifth clade is represented by *C. orinocensis* and the sixth by *C. ocellaris* (WILLIS et al., 2007).

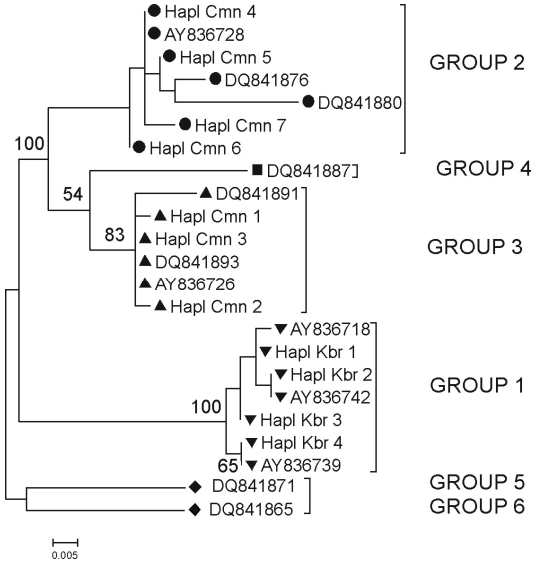

Figure 2. Maximum-likelihood phylogenetic tree generated from partial sequences of the mtDNA control region (*D-loop* gene) of the species shown in Table 1. GROUP 1: ▼ *C. kelberi*: Tocantins river, Araguaia river, upper Paraná river floodplain and Itaipu reservoir. GROUP 2: ● *C. monoculus*: Capivara reservoir, Taquaruçu reservoir, Rosana reservoir, Tapajós river and Xingu river. GROUP 3: ▲ *C.monoculus*: Solimões river, Capivara reservoir, Taquaruçu reservoir, Rosana reservoir, Tapajós river and Xingu river. GROUP 4: ■ *C. monoculus*: Madeira river, Orinoco river and Negro river. GROUP 5: ◆ *C. ocellaris*. GROUP 6: ◆ *C. orinocensis*.

Within each of the six clades, there was a low genetic variation (Table 4); or no index computed (N/C) - since only one individual formed the clades used as external group. Clade four had the highest genetic diversity; composed of samples collected in Madeira, Orinoco and Negro rivers by Willis et al. (2007). The difference of 2.3% can be explained because the groups of the present study were made according to the analysis of Willis et al. (2007), which also found three groups of *C. monoculus*. The smallest genetic distance was observed within the group one of *C. kelberi*, which highlights the high degree of relatedness between specimens of this species.

The largest distance was found between the clades four of *C. monoculus* and *C. kelberi* (0.089) and the smallest distance between the clades three and four of *C. monoculus* (0.041) (Table 4).

The *p*-distance matrix was used to construct a scatterplot of the main coordinates of six groups of *Cichla* (Figure 3). *C. kelberi* formed an isolated group, detected along the axis one. *C. monoculus* formed two groups, but only detected by the dispersion in the axis two. Moreover, the species *C. orinocensis* and *C. ocellaris* showed no marked differences in relation to *C. monoculus*.

Discussion

The obtained results evidenced that four haplotypes of *C. monoculus* presented in the Paraná river have a high haplotype frequency, showing a similarity with the haplotypes of the samples collected in the rivers Tapajós and Solimões. The first haplotype was not found in Porto Rico. The third and fourth haplotypes differ by having a deletion of thymine regarding the first and the second haplotypes. The study performed by Oliveira et al. (2006) showed that in the samples gathered in Capivara reservoir, there was one haplotype shared with the native population of the Amazonas river. Then, Oliveira et al. (2008) analyzed sixty-five specimens of *Cichla* of the Upper Paraná river and Amazon basins by employing the non-transcribed region of 5S rDNA gene to obtain specific markers for related species, and observed that all specimens of *C. monoculus* of the Amazon river basin and the upper Paraná river basin presented the same amplified fragments. This confirms the results herein registered, i.e., the populations introduced in the Paranapanema river were possibly specimens from the Solimões and Tapajós rivers.

According to Almeida-Ferreira et al. (2011), *Cichla monoculus* Spix, 1831 was the first species identified in the Paraná river in 1986. However, according to Oliveira et al. (2006), the genus *Cichla* is represented by two species (*C. kelberi* and *C. piquiti*) erroneously identified as *C.monoculus* and *Cichla* sp. Oliveira et al. (2008) identified *C. kelberi* and *C. piquiti* in the Paraná river, in accordance with the data reported by Kullander and Ferreira (2006), and identified haplotypes from the Solimões river in samples of *C. monoculus* collected in the Paranapanema river. In the present study, we recorded two species of *Cichla* (*C. monoculus* and *C. kelberi*) in the study areas. A small number of samples of *C. kelberi* were taken from the Taquaruçu reservoir, indicating the need for further sampling or that the species was recently introduced. Agostinho et al. (2007) stated that the species of peacock bass appear to have established in the basin of the Paraná river by other mechanisms other than the restocking programs developed by the electricity sector.

The peacock bass is recorded for at least 35% of 71 Brazilian reservoirs reviewed by Agostinho et al. (2007). These authors emphasized that these species are present in these reservoirs for decades and have established viable populations, and in some, dominate the assemblies, both in number and biomass. The first introduction of the peacock bass in the Paranapanema river was in Capivara reservoir, in 1997 (ORSI; AGOSTINHO, 1999), and the first confirmed capture took place in 1999 (SHIBATTA et al., 2002). Currently, it is also found in Rosana and Taquaruçu reservoirs, located downstream. The peacock bass is present in practically all reservoirs located along the rivers Grande, Tietê and Paraná. In the reservoirs of the Paranapanema river, the percentage of occurrence was lower (25%), although samplings performed in the Capivara (HOFFMANN et al., 2005) and Taquaruçu reservoirs (BRITTO; CARVALHO, 2006) showed that the distribution in this basins has increased rapidly. In smaller sub-basins, usually with small reservoirs, the occurrence of this species is more restricted (AGOSTINHO et al., 2007).

Table 4. Genetic distance (P) within and between clades of *Cichla* haplotypes from invasive and native populations, based on the hypervariable sequence of the mtDNA control region.

Cichla haplotypes	2. *C. monoculus*	1. *C. kelberi*	3. *C. monoculus*	4. *C. monoculus*	5. *C. orinocensis*	6. *C. ocellaris*
2. *C. monoculus*	0.010					
1. *C. kelberi*	0.077	0.006				
3. *C. monoculus*	0.046	0.070	0.011			
4. *C. monoculus*	0.059	0.089	0.041	0.023		
5. *C. orinocensis*	0.067	0.094	0.062	0.072	—	
6. *C. ocellaris*	0.061	0.082	0.061	0.072	0.071	—

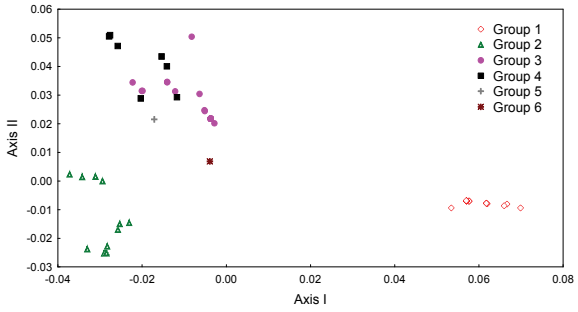

Figure 3. Scatterplot of the main coordinates of the six groups of *Cichla*. Group 1: *C. kelberi* from São Felix de Araguaia, Itaipu reservoir, region of Porto Rico, Taquaruçu reservoir and Tocantins basin; Group 2: *C. monoculus* from the reservoirs Rosana, Taquaruçu and Capivara in the Paranapanema river, Paraná river in the region of Porto Rico, Tapajós river and according to Willis et al. (2007) regions of Xingu, Amazonas and Tapajós; Group 3: *C. monoculus* from Solimões, Taquaruçu, Capivara, Rosana and according to Willis et al. (2007) from Solimões, Xingu and Tapajós; Group 4: *C. monoculus* according to Willis et al. (2007) from Madeira, Orinoco and Negro rivers; Group 5: *C. orinocensis* and Group 6: *C. ocellaris*.

The data obtained showed a strong evidence that *Cichla monoculus* was introduced in the Capivara reservoir as described by Oliveira et al. (2006, 2008), from the region of Manaus, and suggest the dispersion to the reservoirs located downstream (Taquaruçu and Rosana) by sequential occupation according to the models proposed by Heger and Trepl (2003), comprising the phases of establishment, population growth and dispersal of specimens (propagule pressure). This hypothesis is supported by the results found by Luiz et al. (2005) and Pelicice et al. (2005), since samplings undertaken in Rosana reservoir in 2000-2003 had not detected its presence. Moreover, the occurrence of the same haplotypes in the three reservoirs suggests the occurrence of a single introduction.

Conclusion

The present study proposed the genetic identification of the peacock bass and assisted the understanding of Neotropical invasions on the Parana river, Brazil. Additionally, an experimental study in genetics population would be useful to test the gene flow and the asymmetric patterns of migration in order to analyze and identify what is happening with dispersal of *Cichla* after the introduction into the Capivara reservoir.

Acknowledgements

The authors wish to thank the Research Nucleus in Limnology, Ichthyology and Aquaculture (NUPELIA), the Complex of Centrals of Research Support (COMCAP) and the Coordination for the Improvement of Higher Education Personnel (CAPES).

References

AGOSTINHO, A. A.; GOMES, L. C.; PELICICE, F. **Ecologia e manejo de recursos pesqueiros em reservatórios do Brasil**. Maringá: Eduem, 2007.

ALMEIDA-FERREIRA, G. C.; OLIVEIRA, A. V.; PRIOLI, A. J.; PRIOLI, S. M. Spar genetic analysis of two invasive species of *Cichla* (Tucunaré) (Perciformes: Cichlidae) in the Paraná river basin. **Acta Scientiarum. Biological Sciences**, v. 33, n. 1, p. 79-85, 2011.

BRITTO, S. G.; CARVALHO, E. D. Ecological attributes of fish fauna in the Taquaruçu reservoir, Paranapanema river (Upper Paraná, Brazil): composition and spatial distribution. **Acta Limnologica Brasiliensis**, v. 18, n. 18, p. 377-378, 2006.

CARVALHO, D. C.; OLIVEIRA, D. A. A.; DOS SANTOS, J. E.; TESKE, P.; BEHEREGARAY, L. B.; SCHNEIDER, H.; SAMPAIO, I. Genetic characterization of native and introduced populations of the neotropical cichlid genus *Cichla* in Brazil. **Genetics and Molecular Biology**, v. 32, n. 3, p. 601-607, 2009.

CRONIN, M. A.; SPEARMAN, W. J.; WILMOT, R. L.; PATTON, J. C.; BICKHAM, J. W. Mitochondrial DNA variation in chinook (*Oncorhynchus tshawytscha*) and chum salmon (*O. keta*) detected by restriction enzyme analysis of Polymerase Chain Reaction (PCR) products. **Canadian Journal of Fisheries and Aquatic Sciences**, v. 50, n. 4, p. 708-715, 1993.

ESPÍNOLA, L. A.; MINTE-VERA, C. V.; JÚLIO, J. H. F. Invasibility of reservoirs in the Paraná Basin, Brazil, to *Cichla kelberi* and Ferreira, 2006. **Biological Invasions**, v.12, p. 1873-1888, 2010.

EXCOFFIER, L.; LAVAL, G.; SCHNEIDER, S. Arlequin ver. 3.1: An integrated software package for population genetics data analysis. **Evolutionary Bioinformatics**, v. 1, n. 1, p. 47-50, 2007.

GRAÇA, W. J.; PAVANELLI, C. S. **Peixes da planície de inundação do alto rio Paraná e áreas adjacentes**. Maringá: Eduem, 2007.

HALL, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **Nucleic Acids Symposium Series**, v. 41, n. 41, p. 95-98, 1999.

HEGER, T.; TREPL, L. Predicting biological invasions. **Biological Invasions**, v. 5, n. 4, p. 313-321, 2003.

HOFFMANN, A. C.; ORSI, M. L.; SHIBATTA, O. A. Diversidade de peixes do reservatório da UHE Escola Engenharia Mackenzie (Capivara), Rio Paranapanema, bacia do alto rio Paraná, Brasil, e a importância dos grandes tributários na sua manutenção. **Iheringia. Série Zoologia**, v. 95, n. 3, p. 319-325, 2005.

HUELSENBECK, J. P.; RONQUIST, F.; NIELSEN, R.; BOLLBACK, J. P. Bayesian inference of phylogeny and its impact on evolutionary biology. **Science**, v. 294, n. 5550, p. 2310-2314, 2001.

KULLANDER, S. O.; FERREIRA, E. J. G. A review of the South American cichlid genus *Cichla*, with description of nine new species (Teleostei, Cichlidae). **Ichthyological Exploration of Freshwaters**, v. 17, n. 4, p. 289-397, 2006.

LIBRADO, P.; ROZAS, J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. **Bioinformatics**, v. 25, n. 11, p. 451-1452, 2009.

LUIZ, E. A.; PETRY, A. C.; PAVANELLI, C. S.; JULIO, J. H. F.; LATINI, J. D.; DOMINGUES, W. M. As assembléias de peixes de reservatórios hidrelétricos do estado do Paraná e bacias limítrofes. In: RODRIGUES, L.; THOMAZ, S. M.; AGOSTINHO, A. A.; GOMES, L. C. (Ed.). **Biocenoses em reservatórios: padrões espaciais e temporais**. São Carlos: RiMa, 2005. p. 169-184.

MEYER, A.; KOCHER, T. D.; BASASIBWAKI, P. Monophyletic origin of Victoria cichlid fish suggested by mitochondrial DNA sequences. **Nature**, v. 347, n. 6293, p. 550-553, 1990.

MONESI, N.; JACOBS-LORENA, M.; PAÇÓ-LARSON, M. L. The DNA puff gene BhC4-1 of *Bradysia higida* is specifically transcribed early prepupal salivary glands of *Drosophila melanogaster*. **Chromosoma,** v. 107, n. 8, p. 559-569, 1998.

OLIVEIRA, A. V.; PRIOLI, A. J.; PRIOLI, S. M.; BIGNOTTO, T. S.; JÚLIO, J. H. F.; CARRER, H.; AGOSTINHO, C. S.; PRIOLI, L. M. Genetic diversity of invasive and native *Cichla* (Pisces: Perciformes) populations in Brazil with evidence of interspecific hybridization. **Journal of Fish Biology**, v. 69, n. 69, p. 260-277, 2006.

OLIVEIRA, V. F.; OLIVEIRA, A. V.; PRIOLI, A. J.; PRIOLI, S. M. Obtaining 5S rDNA molecular markers for native and invasive *Cichla* populations (Perciformes-Cichlidae), in Brazil. **Acta Scientiarum. Biological Sciences**, v. 30, n. 1, p. 83-89, 2008.

ORSI, M. L.; AGOSTINHO, A. A. Introdução de espécies de peixes por escapes acidentais de tanques de cultivo em rios da bacia do Rio Paraná, Brasil. **Revista Brasileira de Zoologia**, v. 16, n. 2, p. 557-560, 1999.

PELICICE, F. M.; AGOSTINHO, A. A.; THOMAZ, S. M. Fish assemblages associated with *Egeria* in a tropical reservoir: investigating the effects of plant biomass and diel period. **Acta Oecologica**, v. 27, n. 1, p. 9-16, 2005.

PRIOLI, S. M. A. P.; PRIOLI, A. J.; JÚLIO, J. H. F.; PAVANELLI, C. S.; OLIVEIRA, A. V.; CARRER, H.; CARRARO, D. M.; PRIOLI, L. M. Identification of *Astyanax altiparanae* (Teleostei, Characidae) in the Iguaçu river, Brazil, based on mitochondrial DNA and RAPD markers. **Genetics and Molecular Biology**, v. 25, n. 4, p. 421-430, 2002.

POSADA, D.; CRANDALL, K. A. Modeltest: testing the model of DNA substitution. **Bioinformatics**, v. 14, n. 9, p. 817-818, 1998.

RENNO, J. F.; HUBERT, N.; TORRICO, J. P.; DUPONCHELLE, F.; NUNEZ, R. J.; GARCIA, D. C.; WILLIS, S. C.; DESMARAIS, E. Phylogeography of *Cichla* (Cichlidae) in the upper Madera basin (Bolivian Amazon). **Molecular Phylogenetics and Evolution**, v. 41, n. 2, p. 503-510, 2006.

ROSENTHAL, A.; COUTELLE, O.; CRAXTON, M. Large-scale of DNA sequencing templates by microtitre format PCR. **Nucleic Acids Research**, v. 21, n. 1, p. 173-174, 1993.

SHIBATTA, O. A.; ORSI, M. L.; BENNEMMANN, S. T.; SILVA-SOUZA, A. T. Diversidade e distribuição de peixes na bacia do rio Tibagi. In: MEDRI, M. E.; BIANCHINI, E.; SHIBATTA, O. A.; PIMENTA, J. A. (Ed.). **A bacia do rio Tibagi**. Londrina: Medri, 2002. p. 403-423.

SWOFFORD, D. L. **PAUP**: phylogenetic analysis using parsimony and other methods version 4. Sundeland: Sinauer Associates, Inc. Publisher, 2002.

TAMURA, K.; DUDLEY, J.; NEI, M.; KUMAR, S. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. **Molecular Biology and Evolution**, v. 24, n. 8, p. 1596-1599, 2007.

THOMPSON, J. D.; HIGGINS, D. G.; GIBSON, T. J.; CLUSTAL, W. Improving the sensitivity of progressive multiple sequence alignment through sequencing weighting, position-specific gap penalties and weight matrix choice. **Nucleic Acids Research**, v. 22, n. 22, p. 4673-4680, 1994.

WILLIS, S. C.; NUNES, M. S.; MONTAÑA, C. G.; FARIAS, I. P.; LOVEJOY, N. R. Systematics, biogeography, and evolution of the Neotropical peacock basses *Cichla* (Perciformes: Cichlidae). **Molecular Phylogenetics and Evolution**, v. 44, n. 1, p. 291-307, 2007.

Received on July 19, 2010.
Accepted on May 21, 2012.