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Spar genetic analysis of two invasive species of *Cichla* (Tucunaré) (Perciformes: Cichlidae) in the Paraná river basin

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ABSTRACT. The introduction of exotic species in lake ecosystems has been greatly highlighted in the literature worldwide. Since introduction may threaten diversity of native fish, the issue turns up to be of paramount importance. Ecological effects may be predation, competition, parasitism or genetic, that is, changes in the genetic pool of populations owing to the occurrence of hybrids. Although the Tucunaré fish (*Cichla*) is native to the Amazon region, it can be found in other hydrographic basins in which it has been introduced. RAPD molecular marker research showed that there are two species (*Cichla kelberi* and *C. piquiti*) belonging to the genus *Cichla* in the rivers of the Paraná basin. Different morphotypes in the region may also be due to hybridization. Current research used SPAR molecular markers to confirm the presence of hybrids and the rupture of isolation mechanisms. Seventy-two specimens collected in several sites of the river Paraná and Amazon basins were analyzed. Since exclusive SPAR molecular markers were obtained for *Cichla kelberi* and *C. piquiti* populations, the introduction of the two species in the region has been confirmed. Identification of the markers in specimens of the Paraná river basin confirmed hybridization between these exotic species.

Key words: hybridization, fish introduction, molecular markers.

RESUMO. Análise genética via spar, de duas espécies de *Cichla* (Tucunaré) (Perciformes: Cichlidae) introduzidas na Bacia do rio Paraná. A introdução de espécies exóticas nos ecossistemas lacustres tem sido muito destacada na literatura mundial, pois ameaça a diversidade de peixes nativos, tornando-se uma questão de extrema importância. Os efeitos observados podem ser ecológicos, como predação, competição e parasitismo ou genéticos, como a ocorrência de híbridos. Apesar do peixe tucunaré (*Cichla*) ser nativo da região amazônica, ele pode ser encontrado em outras bacias hidrográficas nas quais foram introduzidos. Estudos utilizando marcadores moleculares RAPD revelaram que existem duas espécies (*Cichla kelberi* e *C. piquiti*) do gênero *Cichla* na bacia do alto rio Paraná e morfotipos diferentes que podem ser devido à hibridização. O presente trabalho utilizou marcadores moleculares SPAR para confirmar a presença de híbridos e os mecanismos de ruptura de isolamento entre essas espécies. Setenta e dois espécimes foram coletados em diversos pontos da bacia do rio Paraná e da bacia Amazônica e foram analisados. Marcadores moleculares SPAR exclusivos foram obtidos para populações de *Cichla kelberi* e *C. piquiti*, confirmando a introdução das duas espécies na região. A identificação dos marcadores diagnósticos em espécimes da bacia do rio Paraná confirmou também a hibridização entre estas espécies exóticas.

Palavras-chave: hibridização, introdução de peixes, marcadores moleculares.

Introduction

The introduction of species of fish in lacustrine ecosystems has been a prominent target in international literature, focusing on the studies that aim at evaluating the causes and effects of these introductions in native aquatic communities. According to Elvira and Almodóvar (2001) the introduction of exotic species and the loss of natural habitats are together the biggest responsible for the extinction of animal species in the last century. Among the vertebrates, the introductions of species of freshwater fish (exotic introductions or local translocations) have been among the most numerous and the endemic species are among the most vulnerable to these events all around the world, having as result its extinction or a significant numbers reduction (CRIVELLI, 1995).

According to Agostinho and Júlio Júnior (1996), Brazil is the country with the greatest number of introductions of species in aquatic environments. During the last decades, the country has been receiving a high number of not native species of fishes (BUCKUP; MENEZES, 2003) and this introduction has been recognized as one of main direct causes of loss of biodiversity (AGOSTINHO et al., 2005). This practical has been carried out for increase of reservoirs fishing production, in grow of species appreciated by its taste and good development, for sportive intentions or accidentally, because of uninformation or escaping of tanks of fish culture. In the Brazilian Northeast, for example, species of the *Cichla* genus have been introduced, successfully, for increase of the reservoirs fishing production (CHELLAPPA et al., 2003).

The upper Paraná river floodplain, an unique ecosystem with more than 250 species of fish reported, have been strongly affected by the introduction of exotic species (AGOSTINHO et al., 2004, 2005). *Cichla kelberi* (KULLANDER; FERREIRA, 2006) was one of the species introduced in the region to be used in sportive fishing. Currently, it is among the most abundant species (AGOSTINHO et al., 2004). It was previously identified in the upper Paraná river basin as *Cichla monoculus* (Spix; Agassiz, 1831). Another species of the same genus introduced from the Araguaia-Tocantins basin was *Cichla piquiti* (KULLANDER; FERREIRA, 2006), previously identified as *Cichla* sp. In nowadays these populations of *Cichla* have established itself in some areas of the basin, including the Paraná river, reservoirs and tributaries. The fishes of this genus have a vast diversity of alimentary habits, including species with predatory habits that feed themselves mainly of small fishes as *Astianax* spp. and *Apistogramma* spp.

The impacts of *Cichla* introduction have been reported for many authors in other countries (KIRCHEIS; STANLEY, 1981; SHAFLAND, 1996) and in Brazil (NOVAES et al., 2004), mainly those related to ecologic effects as competition, predation and parasitism Oliveira et al. (2006) studying *Cichla* populations from the upper Paraná river floodplain, through nuclear and mitochondria's markers, demonstrated that the introduced populations of *Cichla kelberi* and *Cichla piquiti* from the region are crossing and forming natural hybrids. The hybrid populations has been established successfully, are fertile and are backcrossing with the parental species, resulting in a process already advanced of genetic homogenization. An evidence of hybridization among *Cichla* species (*C. monoculus* and *C. temensis*) also has been reported for native natural populations of the Amazon region (ANDRADE et al., 2001; BRINN et al., 2004; TEIXEIRA; OLIVEIRA, 2005).

Molecular tools can be useful in foreseeing the extension of hybridization events and the methodologies based on molecular markers, via Polymerase Chain Reaction (PCR), are presenting itself as an excellent alternative to contribute for the confirmation of these events. One of the used techniques is known as Single Primers Amplifications Reactions (SPAR) or Inter Simple Sequence Repeat (ISSR), which has generated effective molecular markers in plants and animals (GUPTA et al., 1994). The amplification is carried via PCR and the technique peculiarity is the use of only one primer with the repetitive sequence of a micro satellite or SSR (Single Sequence Repeats). Primers SPAR with tetranucleotide sequences show to be efficient in the informative polymorphic intra-specifics standards production and inter-specifics (GUPTA et al., 1994), once they amplify regions between two micro-satellite blocks.

The genetic study about *Cichla* (Tucunaré) populations introduced in the Paraná river basin is really important from the ecological view in supplying data that may help out for a better taxonomic characterization and guide handling practical and ecology studies better adequate of the species of the genus. Thus, the aim of this paper was to find molecular markers SPAR for the different *Cichla* species introduced in the Paraná river basin, besides obtaining data that corroborate the break of isolation mechanisms and the hybridization process already spoken, finding evidences of introgression of nuclear genes in the gene pool of both exotic species.

Material and methods

Collection and preservation of the samples

Samples from the species of the *Cichla* genus was collected in many sites from the upper Paraná river basin, including the upper Paraná river floodplain and the reservoir of the Itaipu hydroelectric power plant, Paranapanema river and Tietê river. Beyond these, samples from the north region of the country (Amazonian basin) were collected, filling a total of 74 individuals of seven populations.

Three species was previously identified as *Cichla kelberi*, *Cichla piquiti* and *Cichla* cf. *monoculus*, being the other specimen considered hybrids according to works carried by Oliveira et al. (2006) (Table 1).

Table 1. Number of collected fish specimens for each *Cichla* species and suspected hybrids *C. kelberi* x *C. piquiti*, in sampling locations.

Locality	Species			
	C. kelberi	Hybrids	*C. piquiti*	*Cichla* cf. *monoculus*
Paranapanema river – Capivara Hydroelectric Plant reservoir – Alvorada do Sul, Paraná State				8
Solimões river – Manaus, Amazonas State				8
Tocantins river – Porto Nacional (Lajeado reservoir), Ipueiras and Pedro Afonso, Tocantins State	7		7	
Tietê river - Mário Lopes Leão Hydroelectric Plant reservoir – Promissão, São Paulo State			7	
Paraná river – upper Paraná river floodplain – Porto Rico and Itaipu reservoir, Paraná State		28	9	

Samples of muscular tissue were removed from each specimen collected, fixed then in commercial ethanol and storage in freezer -20°C.

Total DNA extraction

The methodology used for extraction of DNA was based in phenol/chloroform (MONESI et al., 1998). Samples of muscular tissue removed from each individual were macerated in liquid nitrogen and homogenized in PS buffer (Tris-HC1 0.2 M, EDTA 30 mM, SDS 2% e Sucrose 5%) TH buffer (Tris-HC1 10 mM, NaCl 60 mM, EDTA 10 mM, Sucrose 5%, Spermine 0.15 mM and Spermidine 0.15 mM) pH 8.0 e protease K (20 μg μL^{-1}) for 90 minutes in bain-marie at 37°C. Later, the DNA was purified by extraction with phenol/chloroform (1:1) and chloroform, respectively, and precipitated with saline solution (NaCl 5 M) and cold ethanol. The pellet was suspended in TE buffer (Tris 10 mM, EDTA 1 mM) with RNAse. Gel electrophoresis of agar 1% was used to estimate the present concentration of DNA in each sample, through comparison with DNA of phage λ of known concentration. After quantified the DNA samples was diluted for 5 ng each one, for the application of the technique of biology molecular known as SPAR or ISSR.

Amplification via SPAR-PCR

With the DNA of *Cichla* a test was initially done using primers with repetitive sequences of micro-satellites to select oligonucleotids used in the SPAR analysis. Of the primers tested, six were selected to used in DNA amplification of all individuals collected, being it: $(CCTA)_4$, $(GGAC)_4$, $(TGTC)_4$, $(AACC)_4$, $(GGAC)_3$ A, $(GGAC)_3$ T. These primers provide exclusive molecular markers for each species.

After carried the amplifications, all samples were fractioned in agarose gel electrophoresis 1,4% stained with ethidium bromide, visualized in a transilluminator under UV light and photographed. The size of each fragment gotten was determined through comparison with bands of a pattern marker (Ladder 100 pb – Invitrogen).

Computational analysis of the data

The individuals were compared inside and among populations, being the comparisons made from absence data (0) and presence (1) of band in the gel for each individual. The not tendentious measures of genetic distance of Nei, the diversity Shannon's index and the gene flow (equivalent to the number of migrants by generation (Nm)) among populations were calculated by computational applicatory Popgen version 1.31 (YEH et al., 1999). A matrix of distance among all individuals, going two by two, has been obtained by the similarity index of Nei and Li (1979), using the Rapdplot program (BLACK, 1997). By the fact of the distance of Nei and Li not to be metric, was carried out the Lingoes method (LEGENDRE; ANDERSON, 1999), using the computational applicatory DistPCoA (LEGENDRE; ANDERSON, 1998).

The grouping analysis was carried out using algorithm Upmga (Unweighted Pair Group Methods of Arithmetic Means) (SNEATH; SOKAL, 1973) through MEGA 2.1 program (KUMAR et al., 2001).

Results and discussion

The SPAR technique is being used for many searchers for identification of fish species, since that has been established a pattern of bands specific species (ALBERT et. al., 1999; PRIOLI et. al., 2004). In the present work 74 specimens of the *Cichla* genus was analyzed, from many points of the Paraná river and Amazon basin. The tested and selected primers for DNA amplification via PCR produced different patterns of SPAR fragment totalizing 36 loci. The number of bands generated by primer in all analyzed populations varied from three to nine and the size of these products amplified remained between 400 and 2400 bases pars (bp).

SPAR electrophoretic patterns, represented on Figure 1, show the profile of some individuals analyzed with the primer $(GGAC)_3$ A. It can be observed on the figure the presence of exclusive and monomorphic DNA bands that were used as diagnostic markers of *Cichla kelberi* and *Cichla piquiti*. This marker is present in some individuals of the upper Paraná river floodplain, demonstrating the hybrid condition.

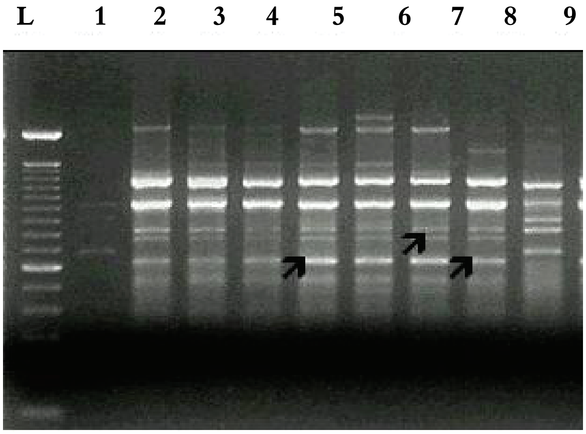

Figure 1. Amplified fragments with the primer $(GGAC)_3A$ from specimens of *Cichla kelberi* (lines 2-7), supposed hybrids (lines 8-13) and *Cichla piquiti* (lines 14-19). The line 1 has the control sample (without DNA) and line L has the fragment markers of molecular weight (Ladder 100 bp, Invitrogen). The black arrows indicate the obtained diagnostic markers for the representatives of *C. kelberi,* as well as its presence into the supposed hybrid. The white arrows indicate the obtained markers for the representatives of *Cichla piquiti*, as well as its presence into the supposed hybrid.

The exclusive DNA fragments and diagnostics were confirmed in native and introduced populations. Introduced populations of *Cichla piquiti* presented in the upper Paraná river floodplain, Itaipu and Promissão reservoir, share exclusive monomorphic nuclear markers with the population of Tocantins river. Hybrid population from the upper Paraná river floodplain also shares monomorphic DNA fragments with the native population of *C. kelberi* native from Tocantins. These results confirm the data obtained by Oliveira et al. (2006) that representative of *Cichla piquiti* and *C. kelberi* from the studied area of the Amazon basin were introduced into the Paraná river basin. Due to the advanced process of genetic homogenization between these species, the presence of the parental species (*C. kelberi* and *C. piquiti*) is less noted at the upper Paraná river floodplain and at the Itaipu reservoir, contrasting with the huge number of hybrid individuals. Populations of *C.* cf. *monoculus* from Manaus and Alvorada do Sul (Capivara reservoir) also shares exclusive monomorphic nuclear markers, what indicates that into the Capivara reservoir had introduction of specimen originated from Manaus area (Amazon basin).

The percentage of polymorphic loci obtained from each population was low with values between 13.89 and 41.67%, indicating a low genetic variability intrapopulation. The great exception occurred in the hybrid population of the upper Paraná river floodplain and at the reservoir of the Itaipu hydroelectric power plant where the individuals showed 66.67% of polymorphic loci.

The estimated genetic diversity by the Shannon's index analyzing all individuals was of 0.4035 ± 0.2753. In isolated populations the index stayed between 0.699 ± 0.1914 and 0.2288 ± 0.2853, reveling also a low genetic diversity, not including again the populations of the floodplain that presented values of 0.3885 ± 0.3020.

Table 2 shows data for Nei's unbiased genetic distances for the seven populations. Estimated values indicate great genetic divergence between the native populations of Tocantins (*Cichla piquiti*) and Alvorada do Sul (*Cichla monoculus*). Some values of genetic distance were low. Representatives of *Cichla monoculus* from Manaus showed small genetic distance comparing to the ones of the same species from Alvorada do Sul (introduced). Estimated values indicate small genetic divergence between the population of native *Cichla piquiti* from Tocantins and the population of the same species introduced into the upper Paraná river floodplain and Itaipu reservoir.

The dendrogram constructed taking the dissimilarities values got into groups the 74 specimens in two different groups (Figure 2). The first one represented by the specimens of *Cichla piquiti* and some hybrid representatives and the second group represented by the specimens of *Cichla kelberi*, *Cichla monoculus* and some hybrid representatives. This pattern confirm the obtained results by Oliveira et al. (2006) in analysis with nuclear and mitochondria's markers.

Table 2. Matrix of the Nei's unbiased genetic distance between *Cichla* populations from the Paraná river basin and Amazon basin: PLA (upper Paraná river floodplain), ITA (Itaipu reservoir), AS (reservoir of the Capivara hydroelectric power plant), PRO (reservoir of Promissão hydroelectric power plant) TO (Tocantins) and MA (Manaus).

Populations	Pla/Ita (hybrids)	Pla/Ita (*C. piquiti)*	AS (*C. monoculus)*	TO (*C. piquiti)*	TO (*C. kelberi)*	MA (*C. monoculus*)	PRO (*C. piquiti)*
Pla/Ita (hybrids)	****						
Pla/Ita (*C. piquiti)*	0.0991	****					
AS (*C. monoculus)*	0.1513	0.2420	****				
TO (*C. piquiti*)	0.2951	0.2269	0.5191	****			
TO (*C. kelberi)*	0.2212	0.0870	0.4721	****			
MA (*C. monoculus*)	0.1173	0.2125	0.0701	0.4248	0.0172	****	
PRO (*C. piquiti)*	0.1858	0.1555	0.3617	0.3927	0.3927	0.3622	****

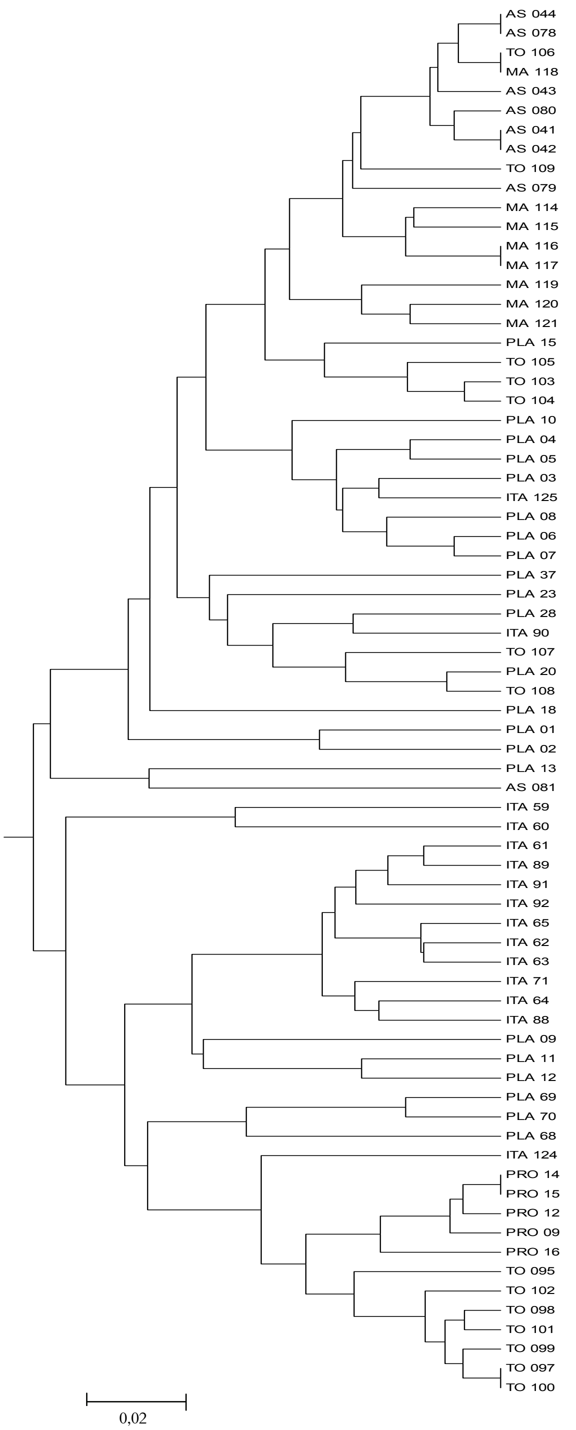

Figure 2. Dendrogram based on the arithmetic complements of coefficients of similarity of Nei and Li, obtained with SPAR markers. The individuals of *Cichla* populations from the Upper Paraná river floodplain (PLA), Itaipu (ITA), Alvorada do Sul (AS), Promissão (PRO), Tocantins (TO) and Manaus (MA) were grouped by the UPGMA method.

However, the first group represented by the dendrogram subdivided itself into two big subgroups, one formed by the hybrid specimens and *Cichla piquiti* from the upper Paraná river floodplain and the other one formed by the hybrid specimens and *Cichla piquiti* from Tocantins, Promissão and from the Itaipu reservoir.

The second group represented by the dendrogram also subdivided itself into two big subgroups, one formed by the hybrid individuals from the upper Paraná river floodplain and the other formed by the specimens of *Cichla monoculus* from Alvorada do Sul and Manaus and *C. kelberi* from Tocantins.

The data present in this paper show strong signs of break of reproductive isolation between species of *Cichla* in the new ambient, resulting in hybridization. The low frequency of parental species in the area reinforces this fact. As hybrids are prevalent and the obtained data indicates that it is about advanced progenies they are probably fertile.

The hybrid representatives present in the studied regions of the Paraná river basin can be the result of break of mechanisms of isolation that existed between the both species (*Cichla kelberi* and *Cichla piquiti*) in native areas and that when introduced in another region, started the hybridization process. Events of hybridization has being reported in regions where the introduced species are genetically compatible with the local species or with other introduced species (ARTHINGTON, 1991; HUBBS, 1955; OLIVEIRA et al., 2002) as is the case of species of *Cichla* that show high karyotypic similarity (2n = 48). Events of hybridization also were observed in works with another species of the genus *Cichla* (*Cichla monoculus* and *Cichla temensis*) that live in simpatry in the Amazon basin (ANDRADE et al., 2001; BRINN et al., 2004; TEIXEIRA; OLIVEIRA, 2005).

Inter-specific hybridization can lead to extinction of local species and the lost of unique genetic pools (PERRY et al., 2002; SCRIBNER et al., 2001). Viable hybrids cause more impacts than sterile hybrids, once they can cross between itself or retro cross with parental species, inserting genes of one species in the gene pool of another species. However, events of introgression can propitiate favorable conditions for a bigger and faster evolution and has a great contribution in gene diversity inside of the species (BERNATCHEZ et al., 1995). As discussed by Smith et al. (2003) the hybridization between cichlids can be more significant with one evolutionary impact than previously assumed.

The biggest treat of introgression is that the gene flow is uninterrupted and the genetic difference between populations will decrease and local adapted stocks will be substituted for big populations more homogeneous. Events of hybridization between exotic species, many times with the introgression of genes, can have serious implications, resulting in ecologic differences and the loss of biodiversity, culminating in big changes in ecosystems of freshwater. In this sense, studies of ecology and biology of species of *Cichla* are necessary, including hybrid populations, for then assess the impacts caused by these exotic populations in the region and its relations with others native populations.

Conclusion

Exclusive SPAR molecular markers were obtained for *Cichla kelberi* and *C. piquiti* populations and the introduction of the two species in the region has been confirmed.

Identification of these markers in specimens of the river Paraná basin confirmed hybridization between these exotic species.

The SPAR molecular markers did prove useful for identifying natural hybrids between *C. kelberi* and *C. piquiti* in the upper Paraná river floodplain.

References

AGOSTINHO, A. A.; JÚLIO JÚNIOR.; H. F. Ameaça ecológica: Peixes de outras águas. **Ciência Hoje**, v. 21, n. 124, p. 36-44, 1996.

AGOSTINHO, A. A.; BINI, L. M.; GOMES, L. C.; JÚLIO JÚNIOR, H. F.; PAVANELLI, C. S.; AGOSTINHO, C. A. Fish Assemblage. In: THOMAZ, S. M.; AGOSTINHO, A. A.; HAHN, N. S. (Ed.). **The upper Parana river and its Floodplain: Physical Aspects, Ecology and Conservation**. Leiden: Backhuys Publishiers, 2004. p. 223-246.

AGOSTINHO, A. A.; THOMAZ, S. M.; GOMES, L. C. Conservation of the biodiversity of Brazil's inland waters. **Conservation Biology**, v. 19, n. 3, p. 646-652, 2005.

ALBERT, J.; FERNANDES-MATIOLI, F. M. C.; ALMEIDA-TOLEDO, L. F. A new specie of *Gymnotus* (Gymnotiformes, Teleostei) from Southeastern Brazil: towards the descontruction of *Gymnotus carapo*. **Copeia**, v. 1, n. 1, p. 85-102, 1999.

ANDRADE, F.; SCHNEIDER, H.; FARIAS, I.; FELDBERG, E.; SAMPAIO, I. Análise filogenética de duas espécies simpátricas de tucunaré (*Cichla*, Perciformes), com registro de hibridização em diferentes pontos da bacia amazônica. **Revista Virtual de Iniciação Acadêmica da UFPA**, v. 1, n. 1, p. 1-11, 2001.

ARTHINGTON, A. H. Ecological and genetic impacts of introduced and translocated freshwater fishes in Australia. **Canadian Journal of Fisheries and Aquatic Sciences**, v. 48, n. 1, p. 33-43, 1991.

BERNATCHEZ, L.; GLÈMET, H.; WILSON, C. C.; DANZMANN, R. G. Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). **Canadian Journal of Fisheries and Aquatic Sciences**, v. 52, n. 1, p. 179-185, 1995.

BLACK, B. **RAPDPLOT 3.0**. Colorado State University, 1997.

BRINN, M. N.; PORTO, J. I.; FELDBERG, E. Karyological evidence for interspecific hybridization between *Cichla* cf. *monoculus* and *C. temensis* (Perciformes, Cichlidae) in the Amazon. **Hereditas**, v. 141, n. 3, p. 252-257, 2004.

BUCKUP, P. A.; MENEZES, A. **Catálogo dos peixes marinhos e de água doce do Brasil.** São Paulo: Museu de Zoologia da USP, 2003.

CHELLAPPA, S.; CÂMARA, M. R.; CHELLAPPA, N. T.; BEVERIDGE, M. C. M.; HUNTINGFORD, F. A. Reproductive ecology of a neotropical cochlid fish, *Cihla monoculus* (Osteichthyes: Cichlidae). **Brazilian Journal of Biology**, v. 63, n.1, p.17-26, 2003.

CRIVELLI, A. J. Are fish introductions a threat to endemic freshwater fishes at the Northern Mediterranean Region? **Biological Conservation**, v. 72, n. 2, p. 311-319, 1995.

ELVIRA, B.; ALMODÓVAR, A. Freshwater fish introductions in Spain: facts and figures at the beginning at the 21st century. **Journal of Fish Biology**, v. 59, p. 323-331, 2001.

GUPTA, M.; CHYI, Y. S.; ROMERO-SEVERSON, J.; OWEN, J. L. Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. **Theoretical and Applied Genetics**, v. 89, n. 7-8, p. 998-1006, 1994.

HUBBS, C. L. Hybridization between fish species in nature. **Systematic Zoology**, v. 4, p. 1-20, 1955.

KIRCHEIS, F. W.; STANLEY, J. C. Theory and practice of forage-fish management in New England. **Transactions American Fisheries Society**, v. 110, n. 6, p.729-737, 1981.

KULLANDER, S. O.; FERREIRA, E. J. G. A review of the South American cichlid genus *Cichla*, with descriptions of nine new species (Teleostei: Cichlidae). **Ichthyologic Exploration of Freshwaters**, v. 17, n. 4, p. 289-398, 2006.

KUMAR, S.; TAMURA, K.; JAKOBSEN, I. B.; NEI, M. **MEGA2**: Molecular Evolutionary Genetics Analysis software. Tempe: Bioinformatics, Arizona State University, 2001.

LEGENDRE, P.; ANDERSON, M. J. **Program DistPCoA**. Montréal: Département de Sciences Biologiques, Université de Montréal, 1998.

LEGENDRE, P.; ANDERSON, M. J. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. **Ecological Monographs**, v. 69, n. 1, p. 1-24, 1999.

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

NEI, M.; LI, W. H. Mathematical model for studying genetic variation in terms of restriction endonucleases. **Proceedings of the National Academy of Sciences**, v. 76, n. 10, p. 5269-5273, 1979.

NOVAES, J. L. C.; CARAMASCHI, E. P.; WINEMILLER, K. O. Feeding of *Cichla monoculus* Spix, 1829 (Teleostei: Cichlidae) during and after reservoir formation in the Tocantins River, Central Brazil. **Acta Limnologica Brasileira**, v. 16, n. 1, p. 41-49, 2004.

OLIVEIRA, A. V.; PRIOLI, A. J.; PRIOLI, S. M. A. P.; PAVANELLI, C. S.; BIGNOTTO, T. S.; JÚLIO JÚNIOR, H. F.; PANARARI, R. S. Diversity and genetic distance in populations of *Steindachnerina* in the upper Paraná river floodplain of Brazil. **Genetica**, v. 115, n. 3, p. 259-267, 2002.

OLIVEIRA, A. V.; PRIOLI, A. J.; PRIOLI, S. M. A. P.; BIGNOTTO, T. S.; JÚLIO JÚNIOR, 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, p. 260-277, 2006.

PERRY, W. L.; LODGE, D. M.; FEDER, J. L. Importance of hybridization between indigenous and nonindingenous freshwater species: an overlooked threat to North American biodiversity. **Systematic Biology**, v. 51, n. 2, p. 255-275, 2002.

PRIOLI, A. J.; LÚCIO, L. C.; MANIGLIA, T. C.; PRIOLI, S. M. A. P.; JÚLIO JÚNIOR.; H. F, PAZZA, R.; CARRER, H.; PRIOLI, L. M. Molecular markers and genetic variability of *Hoplias aff. malabaricus* populations from the upper Paraná river floodplain. In: AGOSTINHO, A. A.; RODRIGUES, L.; GOMES, L. C.; THOMAZ, S. M.; MIRANDA, L. E. (Ed.) **Structure and functioning of the Paraná river and its floodplain**: LTER – Site 6 – (PELD – Sítio 6). Maringá: Eduem. 2004, p. 163-168.

SCRIBNER, K. T.; PAGE, K. S.; BARTRON, M. L. Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. **Reviews in Fish Biology and Fisheries**, v. 10, n. 3, p. 293-323, 2001.

SHAFLAND, P. L. An overview of Florida's introduced butterfly peacock bass (*Cichla ocellaris*) sportfishery. **Natura**, v. 96, p. 26-29, 1996.

SMITH, P. S.; KONINGS, A. B.; KORNFIELD, I. Hybrid origin of a cichlid population in lake Malawi: implications for genetic variation and species diversity. **Molecular Ecology**, v. 12, n. 9, p. 2497-2504, 2003.

SNEATH, P. H.; SOKAL, R. R. **Numerical taxonomy:** The principles and practice of numerical classification. San Francisco: W.H. Freeman, 1973.

TEIXEIRA, A. S.; OLIVEIRA, S. S. Evidence for a natural hybrid of peacock bass (*Cichla* cf. *monoculus* vs. *Cichla temensis*) based on esterase electrophoretic patterns. **Genetics and Molecular Research**, v. 4, n. 1, p. 74-83, 2005.

YEH, F. C.; BOYLE, T.; YE, Z.; XIYAN, J. M. **POPGENE VERSION 1.31**: Microsoft Window-based freeware for population genetic analysis. Alberta: University of Alberta and Center for International Forestry Research, 1999.

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