Obtaining 5S rDNA molecular markers for native and invasive *Cichla* populations (Perciformes – Cichlidae), in Brazil

Viviane Fátima de Oliveira[1], Alessandra Valéria de Oliveira[1*], Alberto José Prioli[2,3] and Sônia Maria Alves Pinto Prioli[2,3]

*[1]Centro Universitário de Maringá, Av. Guedner, 1610, Jd. Aclimação, 87050-390 Maringá, Paraná, Brazil. [2]Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. [3]Núcleo de Pesquisas em Limnologia Ictiologia e Aqüicultura, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: alessoli@cesumar.br*

ABSTRACT. The 5S rDNA gene is informative and has high conservation rates along the eukaryotic genome, having unique hereditary characteristics. Molecular studies with the 5S rDNA gene have been carried out with several groups, including some species of fish, aiming at solving phylogenetic relationship problems, ancestral patterns and genetic diversity among groups in natural populations. Species of the *Cichla* genus, introduced in the upper Paraná river basin, present some genetic polymorphisms detected by RAPD and SPAR analyses. These species have been intercrossing and forming viable hybrids, with greater genetic variability. The objective of this work was to standardize the amplification methodology for the non-transcribed regions of 5S rDNA multigenic family of *Cichla*, and to obtain specific markers for parent species that could also be identified in the hybrids. Sixty-five specimens of *Cichla* collected from the upper Paraná river and Amazon basins were analyzed. Although molecular markers that could be useful in the identification of hybrids were not obtained, genetic molecular 5S rDNA species-specific markers for *Cichla temensis* that can be employed to identify of this species, as well population markers that can be useful in population genetic variability studies, were obtained.

Key words: introduced species, peacock bass, 5S rDNA.

RESUMO. Obtenção de marcadores moleculares 5S rDNA para populações nativas e introduzidas de *Cichla* (Perciformes – Cichlidae), do Brasil. O gene DNAr 5S é informativo e possui altas taxas de conservação ao longo do genoma dos eucariotos, possuindo características únicas que são hereditárias. Estudos moleculares do gene DNAr 5S vem sendo realizados com diversos grupos, inclusive em algumas espécies de peixes, com o intuito de solucionar problemas de relações filogenéticas, padrão de ancestralidade e diversidade genética, entre grupos de populações naturais. Espécies do gênero *Cichla,* introduzidas na bacia do alto rio Paraná, apresentam polimorfismos genéticos, detectados por análise de RAPD e SPAR. Essas espécies estão intercruzando-se e formando híbridos viáveis, com maior variabilidade genética. O objetivo desse trabalho foi padronizar a metodologia de amplificação de regiões não-transcritas da família multigênica rDNA 5S de *Cichla* e obter marcadores específicos para as espécies parentais que pudessem, também, ser identificados nos híbridos. Foram analisados 65 espécimes de *Cichla*, das bacias do alto rio Paraná e Amazônica. Apesar de não se obter marcadores moleculares que pudessem ser úteis à identificação de híbridos, foram obtidos marcadores moleculares genéticos DNAr 5S espécie-específicos para *Cichla temensis*, que podem ser utilizados para identificação de exemplares dessa espécie e, também, marcadores populacionais, que podem ser úteis para estudos de variabilidade genética populacional.

Palavras-chave: espécies introduzidas, tucunaré, DNAr 5S.

Introduction

The upper Paraná river system belongs to Paraná's ichthyic fauna region (Géry, 1969), which includes the Plata, Uruguay, Paraná and Paraguay river systems, representing the second largest hydrographic drainage in South America, with 3.2 million km^2 (Lowe-McConnell, 1987 and 1999). It corresponds to the Paraná river basin located upstream from Sete Quedas (Seven Falls – now inundated by the Itaipu Reservoir), including large tributaries such as the Grande, Paranaíba, Tietê and Paranapanema rivers. This region has received, in

the past few years, a great number of introduced species, among them amazonic piscivores, which have been very successful in establishing themselves in the region (Agostinho *et al.*, 2004 and 2005).

The introduction of species in aquatic ecosystems has been documented worldwide, but the impact of their presence in the new environments is not yet completely known. According to Elvira and Almodóvar (2001), the introduction of species and the loss of natural habitats were the most important factors responsible for the extinction of animal species in the last century. The authors highlight that exotic species may affect indigenous species due to competition for resources, predation over the native fauna, the introduction of new pathogens, hybridization with native species or significantly alteration of the habitat, which may happen simultaneously with environmental degradation. As proposed by Agostinho *et al*. (2005), in Brazilian waters, the introduction of fish species has been recognized as one of the main direct causes for biodiversity loss.

Fishes of the *Cichla* Schneider, 1801 genus, popularly known as peacock bass, are among the species that were introduced in many hydrographic basins, including the upper Paraná river basin. *Cichla kelberi* (Kullander and Ferreira, 2006) was one of the species introduced in the region to be used in sport fishing. Nowadays, it is among one of the most abundant species (Agostinho *et al*., 2003 and 2004; Júlio Jr. and Agostinho, 2003). Before that, it was identified in the upper Paraná river as *Cichla* cf. *monoculus* Spix and Agassiz, 1831. Another species of the same genus introduced in the Araguaia-Tocantins basin, was *Cichla piquiti* (Kullander and Ferreira, 2006), previously identified as *Cichla* sp. Nowadays, populations of *Cichla* have been establishing themselves in many basin areas, including the Paraná river, reservoirs and tributaries. Oliveira *et al*. (2006), when studying upper Paraná river floodplain populations of *Cichla,* through nuclear and mitochondrial markers, observed that introduced populations of *Cichla kelberi* and *Cichla piquiti* in the region are crossing and forming natural hybrids. Hybrid populations have been establishing themselves with great success; they are fertile and are retro-crossing with parent species, resulting in an already advanced process of genetic homogenization. Evidences of hybridization among species of *Cichla* (*C. monoculus* and *C. temensis*) have also been reported in natural native populations of the Amazon region (Andrade *et al*., 2001; Brinn *et al*., 2004; Teixeira and Oliveira, 2005).

Hybridization among introduced fishes involves genetic risks that vary according to each population. Besides representing a serious mechanism in the extinction of species, and a threat to the integrity of unique genetic pools (Scribner *et al*., 2001; Perry *et al*., 2002), it may also lead to the origin of much better adapted, more vigorous, and also more aggressive lineages (Arthington, 1991). As in many areas of the upper Paraná river basin, the hybrid populations of *Cichla* became very well established; they may be competing for space, food resources and spawning sites with other native species. The possibility of a dominant hybrid population, perhaps through adaptive advantages, will have to be investigated.

Thus, genetic studies with *Cichla* (peacock bass) populations introduced in the Paraná river basin are very important from an ecological point of view, since they provide data that can be used for a better taxonomic characterization, as well as leading to handling practices guidelines and ecology studies more suitable to the genus species and the hybrid populations.

Recent advances in molecular biology techniques have allowed studies on the genetic structuring of populations, the analysis of polymorphisms, and the identification of natural hybrids. Among these techniques, the 5S rDNA is an important one, consisting of sequences that codify 5S rDNA and that are separated one from the other by Non-Transcribed Spacers – NTSs (Martins and Wasko, 2004).

Data obtained on the presence of variant classes of 5S rDNA are rather common, and this may be detected in distinct portions of the genome (multiple and different chromosome localizations), forming differential sets of these sequences repeated in tandem (Martins and Wasko, 2004). Many studied organisms, especially fishes, present two or more differential classes of these sequences, which indicates the intense dynamism of these repeating elements (Messias *et al*., 2003; Martins and Wasko, 2004).

Studies on these sequences in several organisms have demonstrated that, predominantly, the 5S rDNA variations are present in the NTSs and are characterized as the result of insertions, deletions, mini-repetitions and pseudo-genes in these regions. As they are not directly involved in the transcription, they do not suffer from selection pressures, and these variations may be species-specific, suggesting a rapid evolution of these sequences (Suzuki *et al*., 1996).

The use of 5S rDNA repetitions presents some advantages over other available markers. Since the presence of codifying sequences, conserved, flanking NTSs variable regions, favor employment of the PCR

(Polymerase Chain Reaction) technique and, consequently, NTSs isolation of most different species without any previous knowledge of the genome of the species in question (Martins and Wasko, 2004).

The objective of this work was to standardize the amplification methodology for the non-transcribed regions of 5S rDNA multigenic family of *Cichla*, and to obtain specific markers for parent species that could also be identified in the hybrids.

Material and methods

Specimens of *Cichla kelberi* and *C. piquiti* were collected from different points of the upper Paraná river basin (including the floodplain region of the upper Paraná river, Itaipu Hydroelectric Plant Reservoir and Tietê river) and the Amazon basin (Tocantins river). In addition, specimens of other species of the *Cichla* genus (*Cichla temensis* and *C. monoculus*), from the Amazon basin (São Lucas do Rio Verde fishing pond – Mato Grosso State and Solimões river) and the Capivara Hydroelectric Plant Reservoir – Paranapanema river, were also collected, resulting in a total of 65 individuals from seven localities (Figure 1).

Figure 1. Sampling places where species of *Cichla* were collected: 1 to 4: upper Paraná river basin, where species of *Cichla* were introduced. 5 to 7: Amazon basin, where species of *Cichla* are native. 1 – upper Paraná river floodplain, Paraná State; 2 – Paraná river – Itaipu Reservoir, Paraná State; 3 – Paranapanema river – Capivara Hydroelectric Plant Reservoir – Alvorada do Sul, Paraná State; 4 – Tietê river – Mário Lopes Leão Hydroelectric Plant Reservoir – Promissão, São Paulo State; 5 – Tocantins river – Porto Nacional (Lajeado Reservoir), Ipueiras and Pedro Afonso – Tocantins State, 6 – Solimões river – Manaus – Amazonas State; 7 – São Lucas do Rio Verde fishing pond – Mato Grosso State.

Cichla specimens from the Capivara Hydroelectric Plant Reservoir – Paranapanema river and the Solimões river (Amazon basin) were identified as *Cichla* cf. *monoculus*, according to Kullander and Ferreira (2006). Some specimens of the upper Paraná river basin were considered to be hybrids, according to assays carried out with RAPD by Oliveira *et al*. (2006).

Table 1 shows the number of collected specimens for each *Cichla* species and suspected hybrids.

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

Locations	Species				
	C. kelberi	Hybrids	*C. piquiti*	*Cichla* cf. *monoculus*	*C. temensis*
Paranapanema river – Capivara Hydroelectric Plant Reservoir – Alvorada do Sul, Paraná State				7	
Solimões river – Manaus, Amazonas State				6	
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		16	11		
São Lucas do Rio Verde fishing pond – Mato Grosso State					4

From each individual, samples of muscular tissue were collected, which were then fixed in commercial ethyl alcohol and stored in a freezer at -20°C.

The methodology used for the extraction of DNA was totally based on phenol/chloroform (Monesi *et al.*, 1998). The muscular tissue samples were macerated in liquid nitrogen and, to the resulting extract of this mechanical action were added PS buffer (Tris-HCl 0.2 M, EDTA 30 mM, SDS 2% and Saccharose 5%), TH buffer (Tris-HCl 10 mM, NaCl 60 mM, EDTA 10 mM, Saccharose 5%, Spermine 0.15 mM and Spermidine 0.15 mM) pH 8.0 and proteinase K (20 $\mu g\ \mu L^{-1}$) for 90 minutes in a water bath at 37°C. After that, the DNA was purified by extraction with phenol/chloroform (1:1) and chloroform, respectively, and precipitated with saline solution (NaCl 5 M) and cold absolute ethanol. The pellet was re-suspended in TE buffer (Tris 10 mM, EDTA 1 mM) with RNAse. For quantifying the DNA concentration present in each sample, electrophoresis in 1% agar gel was employed by comparison to λ phago DNA with a known concentration. After being quantified, the DNA samples were diluted to 5 ng each, in order to apply the molecular biology technique known as 5S ribosomal DNA.

The 5S rDNA spacer was amplified with primers 5S1 and 5S2, as described by Pendás *et al.* (1995). The amplification reaction mix had in its composition Tris-KCl buffer (Tris-HCl 20 mM pH 8.4 and KCl 50 mM), $MgCl_2$ 2 mM, primer 5S1 0,46 μM, primer 5S2 0.46 μM, dNTP 0.19 mM, *Taq* DNA polymerase 1 U/reaction (Invitrogen), DNA (10 ng) and enough water to fill 13 μL. The amplification reactions were carried out in a MJ Research Inc. Thermocycler programmed for 1 cycle of 4 min. at 92°C, 40 cycles of 1 min at 92°C, 1 min 30 s at 40°C and 2 min. at 72°C. Immediately after the last amplification cycle, the reaction mix was kept during 5 min. at 72°C and cooled during 20 min. at 20°C. Negative controls, without DNA, were included in each set of amplification.

After the amplifications were carried out, all samples were fractioned in 1.4%, agar gel stained with ethidium bromide, visualized in a transilluminator under UV light and photographed. The size of each fragment obtained was determined by the comparison with bands of a standard marker (*Ladder* 100 bp - Invitrogen).

Results and discussion

The non-transcribed region of the 5S rDNA was amplified by PCR from samples of *Cicha kelberi*, *Cichla* cf. *monoculus*, *Cichla piquiti*, *Cichla temensis* and likely hybrids from the upper Paraná river and Amazon river basins, comprising 65 individuals.

The number of clear and reproducible bands obtained by this molecular marker in each species varied from one to four, and the size of the amplified fragment was between 410 to 810 base pairs (Table 2). All individuals analyzed presented a band with a size of 650 bp, this fragment being a marker for the *Cichla* genus.

The 5S rDNA non-transcribed region presents high rates of substitutions, being a suitable molecular marker for population analysis (Martins and Wasko, 2004). In this study, polymorphisms were observed in relation to the size of amplified products in different populations and species of *Cichla*. With this technique, it was possible to obtain species-specific bands that differed in size of base pairs. All specimens of *C.* cf. *monoculus* and *C. kelberi*

from the Amazon river basin and the upper Paraná river basin presented fragments of 650 bp and 410 bp. However, two specimens from Tocantins (*C. kelberi*), presented four bands; in addition to the fragments of 650 bp and 410 bp, they presented fragments of 450 and 810 bp (Specimen 103 - Figure 2 and specimen 104 – Figure 3).

Table 2. Approximate size, in number of base pairs (bp), of DNA fragments obtained by 5S rDNA molecular marker, from *Cichla* species introduced in the upper Paraná and native to the Amazon hydrographic basins.

Locations	Species				
	C. kelberi	Hybrids	*C. piquiti*	*Cichla* cf. *monoculus*	*C. temensis*
Paranapanema river – Capivara Hydroelectric Plant Reservoir – Alvorada do Sul, Paraná State				410/650	
Solimões river – Manaus, Amazonas State				410/650	
Tocantins river – Porto Nacional (Lajeado Reservoir), Ipueiras and Pedro Afonso, Tocantins State	410/450 650/810		410/450 650		
Tietê river - Mário Lopes Leão Hydroelectric Plant Reservoir – Promissão, São Paulo State			650		
Paraná river – upper Paraná river floodplain – Porto Rico	410/650	410/450 500/650	410/650		
Paraná river – Itaipu Reservoir, Paraná State		410/450 500/650	450/500 650		
São Lucas do Rio Verde fishing pond – Mato Grosso State					410/430 650

According to the data obtained by Oliveira *et al.* (2006) and Almeida (2005), the *Cichla* representatives from the Paranapanema river probably had their origin in individuals from native populations of Manaus. Data obtained in the present work corroborate this hypothesis, as no one singular fragment of Tocantins populations was identified in *C.* cf. *monoculus* in the Paranapanema river population (Capivara Reservoir). The fact that the fragment of 450 bp is present in native populations of Tocantins, and in samples of *Cichla* from the upper Paraná river floodplain, also shows the possibility of introductions from this Amazon basin region on the plains, again confirming data obtained previously.

Three specimens of *C. temensis* from Mato Grosso featured three bands with sizes of 650 bp, 430 bp and 410 bp (Figures 2 and 3). The band of 430 bp constitutes a molecular marker of the species.

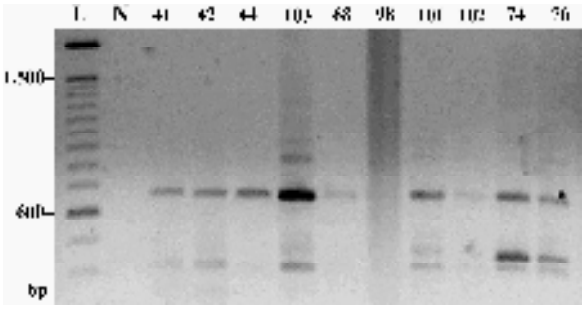

Figure 2. DNA fragments amplified with primers 5S1 and 5S2. Agar gel 1.4%. L = ladder 100 bp; N = negative control. Specimens 41, 42 and 44 – *Cichla* cf. *monoculus* (Capivara Reservoir – Paranapanema river); specimen 103 – *Cichla kelberi* (Amazon basin – Tocantins river); specimen 68 – *Cichla piquiti* (upper Paraná river floodplain); specimens 98, 101 and 102 – *Cichla piquiti* (Amazon basin – Tocantins river); specimens 74 and 76 – *Cichla temensis* (Mato Grosso).

Three specimens of *Cichla piquiti* from the upper Paraná river floodplain and seven specimens from the Tocantins river featured 2 bands of 650 bp and 410 bp. Six specimens of *Cichla piquiti* from the Itaipu Reservoir and seven specimens from Promissão in the Tietê river featured only one band of 650 bp. Only one specimen from the Tocantins river region featured a fragment of 450 bp, shared by two specimens from the Itaipu Reservoir, again indicating introductions in the reservoir from the Amazon basin. These specimens from Itaipu also presented a singular fragment of 500 bp (Figure 3).

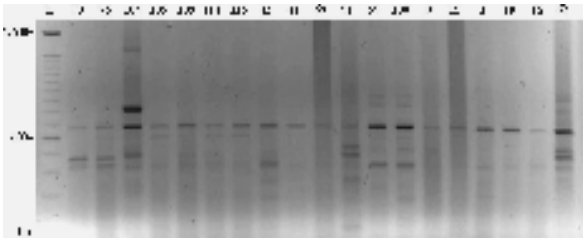

Figure 3. DNA fragments amplified with primers 5S1 and 5S2. Agar gel 1.4%. L = ladder 100 bp; specimens 73 and 76 - *Cichla temensis* (Mato Grosso); specimens 104, 105 and 109 - *Cichla kelberi* (Amazon basin – Tocantins river); individuals 114 and 115 – *Cichla* cf. *monoculus* (Amazon basin – Manaus – Solimões river); specimens 42 and 44 – *Cichla* cf. *monoculus* (Capivara Reservoir – Paranapanema river); specimens 69 and 71 – *Cichla piquiti* (upper Paraná river floodplain and Itaipu Reservoir, respectively); specimens 97 and 100 – *Cichla piquiti* (Amazon basin – Tocantins river); specimens 9 and 15 – *Cichla piquiti* (Promissão – Tietê river); specimens 1, 18, P2 and 62 – likely hybrids (upper Paraná river floodplain and Itaipu Reservoir, respectively).

Some likely hybrids from the upper Paraná river floodplain and Itaipu Reservoir featured two bands with sizes of 650 bp and 410 bp, other specimens featured four bands with 650 bp, 500 bp, 450 bp and 410 bp (Figure 3).

Several genetic and cytogenetic studies on fishes

reveal that the 5S rDNA presents a greater diversity of copies and arrangements in the genome, allowing the distinction between species (Alves, 2005). Pendás *et al*. (1995), when studying the Atlantic Salmon (*Salmo salar*) and the Rainbow Trout (*Oncorhynchus mykiss*), found different sizes of amplified fragments of 5S rDNA, which were useful for identifying the species in question. Perioto (2004) also identified the presence of species-specific bands in different cytotypes of *Hoplias malabaricus* from the upper Paraná river floodplain. In studies with Saint Peter's fish *Oreochromis niloticus, Oreochromis mossambicus, Tilapia rendalli* and the hybrid *O. urolepis*, Alves (2005) obtained species-specific fragments that could be used for the distinction of species and the identification of hybrids.

In the present study, a species-specific molecular marker of 430 bp in *Cichla temensis* was obtained. This fragment may be applied for distinguishing *C. temensis* from other *Cichla* species. The detection of this marker is also relevant in the sense that it can also be identified in natural hybrids, as there are records of hybridization involving this species in native regions (Andrade *et al*., 2001; Brinn *et al*., 2004; Teixeira and Oliveira, 2005).

In studies with lambaris (*Astyanax* sp. F, *Psalidodon gymnodontus* with and without lips, and *Psalidodon* sp.), Panarari (2006) amplified the spacing region of the 5S rDNA gene, but species-specific bands were not identified and the electrophoresis profile of the fragments obtained did not show any genetic differentiation between the morphotypes. Despite the presence of exclusive bands in some individuals, such as seen in Tocantins (*Cichla kelberi*, fragment of 810 bp), Mato Grosso (*Cichla temensis*, fragment of 430 bp) and Itaipu/floodplain (*Cichla piquiti* and hybrids, fragment of 500 bp), species-specific bands for *Cichla kelberi* and *C. piquiti* were not obtained in this study.

Conclusion

The 5S rDNA marker did not prove useful for identifying natural hybrids between *C. kelberi* and *C. piquiti* in the upper Paraná river floodplain. The possibility of identifying events of hybridization in the region must be investigated by other methodologies.

However, this molecular marker can be useful for identifying *Cichla temensis* samples, or for population genetic analysis.

References

AGOSTINHO, A.A. *et al.* Allochthonous species in the upper Paraná basin: status and patterns. *In:* JOINT MEETING OF ICHTHYOLOGISTS AND HERPETOLOGISTS, 2003. Manaus. *Abstracts...* Manaus: American Society of Ichthyologists and Herpetologists (ASIH), 2003. p. 5.

AGOSTINHO, A.A. *et al.* Fish Assemblage. *In:* THOMAZ, S.M. *et al.* (Ed.). *The upper Paraná river and its floodplain*: physical aspects, ecology and conservation. Leiden: Backhuys Publishers, 2004. p. 223-246.

AGOSTINHO, A.A. *et al.* Conservation of the biodiversity of Brazil's inland waters. *Conserv. Biol*., Boston, v. 19, p. 646-652, 2005.

ALMEIDA, G. *Análise genética, via SPAR, de duas espécies de Cichla introduzidas na bacia do rio Paraná.* 2005. Monografia (Graduação em Ciências Biológicas)–Centro Universitário de Maringá, Maringá, 2005.

ALVES, F. *Análise da potencialidade das seqüências de DNA ribossômico 5S como marcadores moleculares para espécies de tilápias (Perciformes, Cichlidade)*. 2005. Dissertação (Mestrado em Genética)–Universidade Estadual Paulista, Botucatu, 2005.

ANDRADE, F. *et al.* 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*, Belém, v. 1, p. 1-11, 2001.

ARTHINGTON, A.H. Ecological and genetic impacts of introduced and translocated freshwater fishes in Australia. *Can. J. Fish. Aquat. Sci*., Ottawa, v. 48, n.1, p. 33-43, 1991.

BRINN, M.N.A. *et al.* Karyological evidence for interspecific hybridization between *Cichla* cf. *monoculus* and *C. temensis* (Perciformes, Cichlidae) in the Amazon. *Hereditas*, Lund, v. 141, p. 252-257, 2004.

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

GÉRY, J. The fresh-water fishes of South America. *In:* FITTKAU, E.J. (Ed.). *Biogeography and ecology in South America*. Junk: The Hague, 1969. p. 828-848.

JULIO JR., H.F.; AGOSTINHO, A.A. Introduced species into the upper Paraná river floodplain by elimination of a geographical barrier and stocking programs. *In:* JOINT MEETING OF ICHTHYOLOGISTS AND HERPETOLOGISTS, 2003. Manaus. *Abstracts...* Manaus: American Society of Ichthyologists and Herpetologists (ASIH), 2003. p. 244-245.

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

LOWE-McCONNELL, R.H. *Ecological studies in tropical fish communities*. Cambridge: Univ. Press Cambridge, 1987.

LOWE-McCONNELL, R.H. *Estudos ecológicos de comunidades de peixes tropicais*. São Paulo: Universidade de São Paulo, 1999.

MARTINS, C.; WASKO, A.P. Organization and evolution of 5S ribosomal DNA in the fish genome. *In:* WILLIAMS, C.R. (Ed.). *Focus on Genome Research*. New York: Nova Science Publishers, 2004. p. 335-363.

MESSIAS, L.H.V. *et al.* 5S rDNA organization in the fish

Synbranchus marmoratus (Synbranchidae, Synbranchiformes). *Hereditas,* Lund, v. 138, p. 228-231, 2003.

MONESI, N. *et al.* The DNA puff gene BhC4-1 of *Bradysia hygida* is specifically transcribed in early prepupal salivary glands of *Drosophila melanogaster. Chromosoma,* Berlin, v. 107, p. 559-569, 1998.

OLIVEIRA, A.V. *et al.* Genetic diversity of invasive and native *Cichla* (Pisces: Perciformes) populations in Brazil with evidence of interspecific hybridization. *J. Fish Biol.,* London, v. 69, p. 260-277, 2006.

PANARARI, R.S. *Caracterização molecular de Astyanax sp. F. e do gênero Psalidodon Eigenmann, 1911 (Characiformes, Characidae) da bacia do rio Iguaçu*. 2006. Exame Geral de Qualificação (Doutorado em Ecologia de Ambientes Aquáticos Continentais)-Universidade Estadual de Maringá, Maringá, 2006.

PENDÁS, A. M. *et al.* Applications of 5S rDNA in atlantic salmon, brown trout, and in atlantic salmon x brown trout hybrid identification. *Mol. Ecol.*, Oxford, v. 4, p. 275-276, 1995.

PERIOTO, P.S. *Variabilidade genética do complexo Hoplias* aff. *malabaricus acessada com marcadores moleculares nucleares e mitocondriais.* 2004. Dissertação (Mestrado em Genética e Melhoramento)–Universidade Estadual de Maringá, Maringá, 2004.

PERRY, W.L. *et al.* Importance of hybridization between indigenous and non-indigenous freshwater species: an overlooked threat to North American biodiversity. *Syst. Biol*., Seattle, v. 51, n. 2, p. 255-275, 2002.

SCRIBNER, K.T. *et al.* Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. *Rev. Fish Biol. Fisher.,* Anchorage, v. 10, p. 293-323, 2001.

SUZUKI, H. *et al.* Rat 5S rDNA spacer sequences and chromosomal assignment of the genes to the extreme terminal region of chromosome 19. *Cytogenet. Cell Genet.,* Basel, v. 72, p. 1-4, 1996.

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. *Genet. Mol. Res.*, Ribeirão Preto, v. 4, p. 74-83, 2005.

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