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DNA barcoding for conservation and management of Amazonian commercial fish

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ABSTRACT

DNA barcoding is used to assign a biological specimen to a species. DNA-based procedure has become the preferred forensic tool for criminal prosecution in cases involving the sale of incorrectly identified food. The aim of this work was to develop a DNA-based marker for allowing an accurate and reliable identification of Amazonian fish species of commercial interest. For this purpose, we extracted DNA from fish directly purchased in local markets and identified de *visu* by local experts. We PCR amplified the mitochondrial 12S rRNA and cytochrome oxidase I (COI) genes. Twenty-nine commercial species accounting for most commercial landings in the River Amazon markets were unambiguously identified based on their DNA for the first time. Phylogenetic trees reconstructed based on the sequences of the two mitochondrial genes clustered species in concordance with their taxonomic classification. We illustrated the utility of DNA barcoding demonstrating that the group of fish generically sold as "*Acará*" includes seven different species, which are being exploited together as a single species, thus estimation of exploitation rates was not possible until now. Application of genetic markers for species authentication in markets and control of commercial landings will contribute to recognition of the real fishing targets and to the conservation of fish resources in the Amazon basin.

1. Introduction

The Amazon rain forest extends over an area of 7.8×10^6 km^2 around the main basin of the River Amazon and its tributaries. The Amazon region contains more than half of the world's remaining tropical forests, and some parts have among the greatest concentrations of biodiversity found anywhere on Earth, hosting about a quarter of all global biodiversity (Betts et al., 2008). More than 3000 fish species are estimated to occur in the more than 1000 tributaries of the River Amazon. The largest part of the basin is located in three countries, Peru, Colombia and Brazil, the main area being Brazilian with nine states located along the basin (Rondõnia, Acre, Amazonas, Roraima, Pará, Amapá, Tocantis, Maranhão and Mato Grosso). It harbours a diverse human population distributed in a few big cities and thousands of quasi isolated communities. Local economies of these regions, especially riparian communities, largely depend on river resources. Fishing is a principal generator of income and food for the rural and urban people in the Amazon (Gram et al., 2001; Batista and Petrere Jr., 2007), as fish species are the main source of animal protein in the diet of local inhabitants (Dorea, 2003). Trade of Amazonian fish is mostly restricted to local markets located along the rivers but even so the figures of fish tons can be huge, approx. 260,000 tm annual catch the last years (63% of the total continental Amazonian fisheries), especially in big cities like Manaus and Belem do Pará, with some exports at national level to other Brazilian states. Fishing plays an important role in the region: food, employment, and enormous contribution to the economic development of the region. It is estimated that in the Amazon basin about 130,000 persons are employed in fishing or activities related with the fisheries sector, extraction of fish being the main engine of the local economy (Gram et al., 2001).

The forest biome of Amazonia is one of the Earth's greatest biological treasures and a potential global contributor towards mitigation of climate change (Betts et al., 2008). The Amazon basin has been transformed by humans since the Pre-Columbine era (Heckenberger et al., 2008). It faces now the dual threats of stress from climate change and over-exploitation of natural resources (e.g. Malhi et al., 2008). These problems affect severely the species inhabiting freshwater habitats, especially vulnerable to human activities (Dudgeon et al., 2006). Fisheries can endanger biodiversity in the Amazon sanctuary even more than in marine waters. Continental extractive fishing in the Amazon area is in an expansive process and some hydrobiological resources show signs of excessive exploitation (Queiroz and Sardinha, 1999), like some of the 20 species which are principal subject of fisheries, amongst them *Arapaima gigas*, *Colossoma macropomum*, *Brachyplatystoma rousseauxii* and *Osteoglossum bicirrhosum*, which are experiencing recent declines (Garcia et al., 2009). However, controlling fisheries

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is not easy in a so vast and intricate freshwater system where tropical jungle is the main ecosystem and human populations are isolated along the tributaries, often hundreds of kilometers apart, without other communication than fluvial transport. In practice, restrictive fishing regulations are limited to some areas declared Natural Reserves, like for example limited catch of *Arapaima gigas* in Mamiraua (Viana et al., 2004).

One of the most basic problems for controlling fisheries is undefined or ambiguous identification of fish species. The Amazonian species are commercialized with common names. These names vary among regions along the basin and can be even different in a single locality. For example, the species *Ageneiosus brevifilis* is called indistinctly *Bocudo*, *Mandubé* or *Fidalgo*. In other cases the same generic name, *pacú*, is applied to at least seven different species belonging to different genera and families (Table 1). In absence of a tool for identification of commercial fish products and authentication of trade fish, this is, the simple *who is who* in Amazonian fish markets, quantification of real figures of extractive catch is impossible and exploitation rates cannot be estimated. Species identification is necessary for sustainable exploitation of fishing resources, as mislabeling may hide exploitation of endangered species (Marko et al., 2004). Based on reliable information about actual fish production, regulation of fisheries can be developed and conservative management measures can be undertaken. To identify, inventory and study fishing resources is indispensable for setting conservative management measures for conciliating fisheries and development with conservation of the Amazonian precious biological diversity. Genetics can help in this purpose. First, for knowing exactly what species is being commercialized under a given local name; and second, for providing a tool for authentication of commercial fish products that cannot be morphologically identified, like slices, salted pieces and other commercial seafood variants. The need of genetic authentication tools has been evidenced in practice as mislabeling has been detected in various fish groups employing DNA-based methodology (e.g. Marko et al., 2004; Machado-Schiaffino et al., 2008). This problem can be more serious in regions that are experiencing fast development and will be more affected by global change, as it is the case of Amazon basin in Brazil (Jenkins, 2003), thus it is urgent to apply species-specific genetic tools for recognition of real fishing targets.

Different DNA-based methods have been proposed for fish traceability (Rasmussen and Morrissey, 2008), generally based on PCR amplification of DNA sequences either targeted or anonymous (Davidson, 1998). FINS (Forensically Informative Nucleotide Sequencing) is a procedure for identifying the animal origin of biological specimens (Barlett and Davidson, 1992) and for identifying food, especially fish products. DNA *barcoding* (Toffoli et al., 2008) is essentially a technique that uses short DNA sequences of standardized and agreed-upon position in the genome as molecular diagnostic for species-level identification. DNA barcode sequences are very short relative to the entire genome and can be obtained reasonably quickly and cheaply, being also applied for molecular diagnostic applications in fish (e.g. Wong and Hanner, 2008). In all cases, DNA barcoding is based on the use of a short standard region that enables cost-effective species identification, and is employed now to catalogue the world's biota, including fish: FISH-BOL is the campaign aimed to DNA barcode all fish species (Ward et al., 2009).

In the present study the genetic variability at one molecular marker, 12S rDNA, was studied in 29 of the main Amazonian commercial species for purposes of developing a barcoding tool for identification of trade species. This mitochondrial gene has been used in many previous works of genetic species identification (Hebert et al., 2003; Lavoué and Sullivan, 2004; Toffoli et al., 2008). Therefore, it seems to be a good candidate for this purpose. The cytochrome oxidase I (COI) gene was employed to confirm species identification as a second marker. The utility of the new DNA tool solving uncertainties due to identical local names was assayed in a group of species generically called "*Acarà*" in different river localities. The main contribution of this work to conservation issues is not the technique, as DNA-based procedures have been extensively employed for fish identification (e.g. Barlett and Davidson, 1992; Hubert et al., 2008), but the origin of the samples (from real local markets from the Amazon basin) and the large number of species analyzed. To our knowledge this is the first time that DNA technology is applied for species identification of commercial Amazonian fish.

Table 1
Samples analyzed. Commercial name, name employed in BFS (Brazilian fisheries statistics). Local names, names of the species in local markets where they were purchased (M, Manaus; N, Novo Airão; T, Tapajós; Tf, Tefé). Catch, catch tons in 2006 (% over total Amazonian catch). 12S AN and COI AN: GenBank Accession number for the 12S rDNA and the COI gene sequences obtained for each species in this study.

Commercial name (BFS)	Local names	Scientific name	Catch (%)	12S AN	COI AN
Acará-açú	Acará-açú (M, N), Acará (T),	*Astronotus ocellatus*	Miscellaneous	FJ998284	FJ978036
Acará	Acará (M), Cará (T), Acará reco (N)	*Geophagus proximus*	2121.5 (1.2%)	GQ258750	–
Aracú	Aracú (M), Caracú (M), Aracú liso (M), Aracú listrado (M)	*Schizodon fasciatus*	6183 (3.5%)	FJ549356	FJ440621
Aruaná	Aruaná (M,T), Aruaná plateada (M), Macaco de agua (T)	*Osteoglossum bicirrhosum*	1679 (1%)	FJ549357	FJ418754
Bocudo	Bocudo (M, T), Mandubé (T)	*Ageneiosus brevifilis*	1882.5 (1.1%)	FJ549358	FJ418755
Candirú	Candirú (M, T),	*Cetopsis candiru*	Miscellaneous	FJ978043	FJ418756
Cuiú-cuiú	Cuiu-cuiu (M)	*Oxydoras niger*	635 (0.4%)	FJ998286	FJ978038
Curimatá	Curimatá (M, T, Tf, N),	*Prochilodus nigricans*	13,858 (7.9%)	FJ549359	FJ418758
Dourada	Dourada (M), Dourado (N)	*Brachypatystoma rousseauxii*	14,471 (8.3%)	FJ549360	FJ418759
Filhote	Filhote(M, T)	*Brachyplatystoma filamentosum*	3401 (2%)	FJ978044	FJ418760
Jaraquí	Jaraquí(M,T), Yaraquí (N)	*Semaprochilodus insignis*	16,054 (9.2%)	FJ549361	FJ457765
Mandi	Mandi (M, T)	*Pimelodus blochii*	2615.5 (1.5%)	FJ998287	FJ978039
Matrinxá	Matrinxá (M, T, N)	*Brycon melanopteurs*	4532.5 (2.6%)	FJ998288	FJ978040
Pacu	Pacú (M, T, N)	*Mylossoma duriventre*	9001.5 (5.2%)	FJ978045	–
Pescada	Pescada (M, T, Tf),	*Plagioscion squamosissimus*	10,398 (5.6%)	FJ549363	FJ418762
Piau	Piau (M, T, N)	*Leporinus piau*	2445 (1.4%)	FJ549364	FJ418763
Pintado	Pintado (M, T), Caparari (M, T)	*Pseudoplatystoma trigrinum*	593.5 (0.4%)	FJ998289	FJ978041
Pirapitinga	Pirapitinga (N), Pirapichinga (M,T)	*Piractus brachypomus*	2117 (1.2%)	FJ998290	FJ978042
Pirarara	Pirarara (M, T, N)	*Phractocephalus hemioliopterus*	752.5 (0.4%)	FJ549365	FJ418764
Pirarucú	Pirarucú (M, T, Tf, N)	*Arapaima gigas*	1176.5 (0.7%)	FJ549366	FJ418765
Surubim	Surubim (M, T, N)	*Pseudoplatystoma fasciatum*	5600 (3.2%)	FJ549367	FJ418766
Tambaquí	Tambaquí (M, T, Tf, N)	*Colossoma macropomum*	4313.5 (2.5%)	FJ549368	FJ418767
Tucunaré	Tucunaré(M, T, Tf, N)	*Cichla temensis*	5200 (3%)	FJ549369	FJ440622
TOTAL	23 SPECIES		110221.5 (63%)		