Fisheries Research 125–126 (2012) 300–305

Contents lists available at SciVerse ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Full length article

Detection of hybrids and genetic introgression in wild stocks of two catfish species (Siluriformes: Pimelodidae): The impact of hatcheries in Brazil

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ARTICLE INFO

Article history:
Received 12 August 2011
Received in revised form
30 December 2011
Accepted 25 February 2012

Keywords:
Pseudoplatystoma
Interspecific hybrid
Introgression
Molecular markers
Genetic conservation

ABSTRACT

Molecular markers (Multiplex-PCR and PCR-RFLP of nuclear and mitochondrial genes) were used to assess the frequency of hybrids in wild stocks of two catfish species: *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum*. Analysis of fish from the Paraguay–Parana hydrographic basin (Brazil) revealed a low frequency of hybrids in the Parana River (3.60%), which might be the result of natural hybridisation. However, the frequency of hybrids was higher in the Mogi Guaçu (50.00%) and Aquidauana (30.75%) Rivers, whose aquaculture systems support the majority of Brazil's fish farms, suggesting that the existence of hybrids in wild populations may be due to introduction or escapes from farm stocks. Furthermore, the identification of a post-F1 individual in the Mogi-Guaçu River provides evidence of genetic introgression. These results show that safeguards are needed to ensure the correct handling of hybrids and the protection of native species.

1. Introduction

Despite the common assumption that hybridisation has little influence on speciation and evolution (Barton and Hewitt, 1985; Mayr, 1963), natural interbreeding between different species, which occurs more frequently in fish than in other vertebrates, could in fact be related to the emergence of new evolutionary lineages, speciation and gene flow (Arnold et al., 1999; Arnold and Hodges, 1995; Burke and Arnold, 2001). While hybridisation can occur natural during evolution, human activity has quickly and dramatically increased the rate of hybridisation in plants and animals (Allendorf et al., 2001; Rhymer and Simberloff, 1996). Almost half of the hybridisation events reported in fish species are the direct or indirect result of human activities, including the degradation of the natural environment, the introduction of foreign species and, primarily, the development of artificial interspecific hybridisation programs in aquaculture (Scribner et al., 2001).

There are two major types of hybridisation events that result from anthropogenic actions: crosses between native and introduced species, as has been documented in trout (Rubidge et al., 2001), carps (Lamer and Dolan, 2010) and cichlids (Almeida-Ferreira et al., 2011) and hybrids resulting from induced breeding between different species (artificial hybrids) for aquaculture (Porto-Foresti et al., 2008; Toledo-Filho et al., 1996). The results of contamination of natural aquatic systems by hybrids can lead to serious ecological disturbances, such as habitat modification and changes in species composition (Toledo-Filho et al., 1994,1998). If the hybrids are fertile, problems derived from the potential for genetic introgression arise, including the creation of hybrid swarms and the resulting genetic extinction of native species (Epifanio and Philipp, 2001; Huxel, 1999; Muhlfeld et al., 2009).

In spite of these problems, artificial hybrids make up a substantial portion of economically important fisheries in several countries, including Brazil, mainly because they are easier to breed and offer improved productivity compared to their parental species (Hulata, 2001; Scribner et al., 2001).

The catfish species *Pseudoplatystoma corruscans* (pintado) and *Pseudoplatystoma reticulatum* (cachara) have been extensively interbred to produce many artificial interspecific F1 (first-generation) hybrids known by fish farmers as "ponto e virgula" (Campos, 2010). "Cachapinta" results from a cross between a cachara female and a pintado male, and "pintachara" results from a cross between a pintado female and a cachara male (Porto-Foresti et al., 2008; Prado et al., 2011). These hybrids have normal embryonic development (Faustino et al., 2010) and exhibit advantageous zoological features, such as higher growth rates than their parental

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doi:10.1016/j.fishres.2012.02.030

species (Crepaldi et al., 2006), which has led to the production of F1 lines overtaking the cultivation of native fishes (Campos, 2010). Moreover, farming stocks of these fish are fertile (J.A. Senhorini, personal observation), which confirm the potential ecological and genetic risks that these genetically manipulated individuals present to pure, native populations of *P. corruscans* and *P. reticulatum*.

These species are widely distributed throughout the main Brazilian hydrographic basins, such as the Amazon (only occurs *P. reticulatum*) and São Francisco River basins (restricted to *P. corruscans*). In some rivers of the Paraguay–Parana River Basin, both species can occur in sympatry (Buitrago-Suárez and Burr, 2007; Langeani et al., 2007). Although several ecological disturbances have constantly affected the wild populations in various localities, especially in the Parana River Basin (Mello et al., 2009), and despite the fact that F1 hybrids have extensively been farmed, posing significant risks to the natural environment, there is a lack of detailed biological studies on hybridisation events involving these species and assessment of artificial hybrid stocks and their impact on wild stocks.

In this regard, an important step toward minimising the risks of artificial hybridisation is genetic monitoring, i.e., the use of molecular tools to analyse the genetic integrity of the natural populations, thus enabling the development of management plans for hybrid fish and conservation programs for the parental species (Toledo-Filho et al., 1994,1998). In this study, molecular markers were used to genetically identify samples of *P. corruscans*, *P. reticulatum* and possible hybrid individuals collected from rivers of the Paraguay River sub-basin and Upper Parana River basin (Parana basin, Brazil) and to investigate the occurrence and frequency of hybrids in these wild stocks.

2. Materials and methods

2.1. Samples and DNA extraction

Twenty pintado specimens (*P. corruscans*) and 20 cachara specimens (*P. reticulatum*) were genetically analysed as a control to confirm the specificity of the markers and to compare the analyses of samples collected from the wild environment. These control individuals were obtained from the stocks maintained at the CEPTA/ICMBio (Centro Nacional de Pesquisa e Conservação de Peixes Continentais, Pirassununga, São Paulo State, Brazil).

A total of 186 biological samples (blood and fin fragments) from the catfishes *P. corruscans* and *P. reticulatum* collected from rivers of the Parana hydrographic system (Brazil) in the period from 2003 to 2008 were analysed. Fish were captured from five sites in the Paraguay sub-basin: the Paraguai River (Pg-1); the Miranda River (Pg-2); the Aquidauana River (Pg-3); the "Parque Nacional do Pantanal Matogrossense" region of the Cuiaba River (Cuiaba-Parna) (Pg-4) and in the Nobres City region (Cuiaba-Nobres) (Pg-5). In the Upper Parana basin, the samples were collected from three sites: the Parana River (Pn-1), the Verde River (Pn-2) and the Mogi-Guaçu River (Pn-3) (Fig. 1). Samples from the Paraguay sub-basin have been stored in the fish collection at the Laboratório de Genética de Peixes, UNESP (Bauru, São Paulo State, Brazil), and individuals from the Upper Paraná sub-basin were provided by J. A. Senhorini, from the fish collection at CEPTA/ICMBio (Pirassununga, São Paulo State, Brazil).

Individuals had previously been identified as *P. corruscans* and *P. reticulatum* using some morphological parameters (Table 1). Thus, as most fishes had not been sacrificed, the species were diagnosed mainly according to the external colour and patterns of the skin accordant with Buitrago-Suárez and Burr (2007): *P. reticulatum* with loop-like dark stripes or bars and *P. corruscans* with dark spots. *P. reticulatum* was used according to the nomenclature of Buitrago-Suárez and Burr (2007), but these individuals can also be referred to as *Pseudoplatystoma fasciatum* (*sensu lato*) (Carvalho-Costa et al., 2011).

There are no studies about the morphological identification of *Pseudoplatystoma* hybrids. Thence, fish with ambiguous or intermediate characters (presenting both spots and loops, for example) were provisionally classified as hybrids (Table 1).

DNA extraction was conducted using the Wizard Genomic DNA Purification Kit for Promega according the manufacturer's protocol. DNA quantity was assessed against a molecular marker standard (the Low DNA Mass Ladder from Invitrogen) by electrophoresis on a 1% agarose gel.

2.2. Molecular markers

Molecular identification of wild stocks of *P. corruscans* and *P. reticulatum* was performed through the PCR (Polymerase Chain Reaction) techniques of regions of the nuclear RAG2 and mitochondrial 16S genes, which provides diagnostic electrophoretic fragments for each parental species (Prado et al., 2011). Multiplex-PCR was used first, followed by PCR-RFLP in subsequent analyses for confirmation. DNA samples from the pure parental species were used as controls for reaction specificity in all experiments.

In PCR-RFLP, fragments of the RAG2 and 16S genes were amplified by PCR using the primer pairs *RAG2 Silu* F (Forward) (5′-CCTGAGTGCTACCTTATTCATGGA-3′) and *RAG2 Silu* R (Reverse) (5′-CTTGGGAGGAAGAGACCATC-3′) (Prado et al., 2011) and *16S* F (5′-ACGCCTGTTTATCAAAAACAT-3′) and *16S* R (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi, 1996), respectively. The enzymatic reaction was carried out using Sau96I for the RAG2 gene and SmlI for the 16S gene (Prado et al., 2011). In multiplex-PCR, in addition to the universal primers for each gene, we used the species-specific primers *RAG2 Pc* R (5′-AACTCCAGGTCAATGAGATAAATG-3′) and *RAG2 Pr* R (5′-CAGTTCCAGGTCTCTGTGGTT-3′) for the RAG2 gene and *16S Pc* F (5′-TGACCATAAAGATCCGGCTAT-3′) and *16S Pr* R (5′-TCTTGGTTTTGGGGTTGTTA-3′) for the 16S gene (Prado et al., 2011).

2.3. Data analysis

Initially, individuals were classified as pure *P. corruscans*, pure *P. reticulatum* or hybrids (F1, backcrosses, and more advanced generations of hybrid classes). Specimens carrying markers for the same species at both RAG2 nuclear gene and 16S mitochondrial gene were considered pure. F1 hybrids were defined as those fish that carried alleles from both parental species (i.e., were heterozygous for the nuclear marker) and were classified as "pintachara" or "cachapinta" according to the mitochondrial marker. Post-F1 individuals were those that carried the genotype of one pure species at the nuclear marker and the genotype of the other pure species at the mitochondrial marker.

3. Results

The molecular data revealed hybrids at three of the eight sampled sites. In the Paraguay sub-basin, hybrid specimens were observed in the Aquidauana River (30.75%), while all individuals from Miranda, Cuiaba-Parna, Cuiabá-Nobres and Paraguay River were identified as being purely *P. corruscans* or *P. reticulatum* (Table 1). The analysis of fish originating from the Upper Parana sub-basin revealed hybrid individuals in the Parana (3.90%) and Mogi Guaçu (50.00%) Rivers, but specimens of only *P. corruscans* in the Verde River (Table 1).

All Paraguay sub-basin and Parana River (Upper Parana basin) hybrids were identified as belonging to the "cachapinta" F1 line, as they were heterozygous at the nuclear marker and identical to