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Primers for the amplification of the MHC II beta chain exon 2 in the Atlantic goliath grouper (Epinephelus itajara)

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Abstract :

In the present study we designed a pair of primers to amplify the exon 2 of the MHC II beta chain of the Atlantic goliath grouper, which is responsible for the recognition of pathogenic molecules and the regulation of the immune system. Future analyses of this region may provide an important database to understand the evolutionary processes affecting the populations of the goliath grouper, and to predict the conservation perspectives in the species.

Keywords : Epinephelus itajara, Genetic diversity, MhcEit-DAB

The formation of spawning aggregations in fishes presents opportunities for efficient fishing and removal of significant proportions of a population within short time frames, however such practice might impact negatively these populations, collapsing them through overexploitation (Sadovy and Eklund, 1999; Tobin et al, 2013). Collapsed populations are likely to reduce their evolutionary fitness towards changes in the environment, eg the raise of new pathogens, making those more susceptible to illnesses (Eizaguirre and Lenz, 2010).

Family Epinephelidae is an example of collapsed populations in a global scale, due to its directioned fisheries activities to the spawning aggregations (Sadovy de Mitcheson et al, 2012). The goliath grouper (*Epinephelus itajara,* Lichtenstein, 1822) is the largest bony fish and it is found in the tropical Atlantic Ocean, reaching 25m in length and over 400kg in weight (Sadovy and Eklund, 1999). As a prominent sport angling species, which is also targeted by commercial fishermen, *E. itajara* has been exploited intensively over the past 30 years, and its populations are now in sharp decline (Aguilar-Perera et al, 2009; McClenachan, 2009). The docile behavior, slow growth, formation of spawning aggregations and current genetic data (low variation) seem to

make its populations truly vulnerable to extinction (Frias-Torres 2006; Gerhardinger et al. 2006; Koenig et al. 2007; Silva-Oliveira et al. 2008; Mann et al. 2009).

The genes responsible for the recognition of peptides molecules and the regulation of the immune system form part of the Major Histocompatibility Complex (MHC), which is the most polymorphic region of the vertebrate genome (May and Beebee, 2009). The region of the MHC, which recognizes and binds to antigens (Peptide Binding Region- PBR), is located in MHC class II. This region of the genome is responsible for increasing the host's capacity to identify specific invasive agents (Eizaguirre and Lenz, 2010). Therefore, many molecular studies of vertebrates have focused on the amplification of exon 2 of the DQB genes of the β chain of the molecules of MHC class II, given the importance of this sequence for the immune response and its high degree of polymorphism (Sonsthagen et al, 2014; He et al, 2014).

No data are available on the attempt to the diversity and selection of MHC II-β locus in endangered *E. itajara.* These new data may provide an important database to understand the evolutionary processes affecting the populations of the *E. itajara*, and to predict the conservation perspectives in the species, especially in terms of genetic variability.

The samples analyzed were obtained from four locations along the Atlantic in Brazil: Pará (14), Piauí (08), Rio Grande do Norte (10) and Pernambuco (09). The samples of the tissue and fins were obtained from local fishermen between 2000-2008. Total DNA was isolated from muscles and fins, following the method described by Sambrook et al (1989). These samples were amplified using the Polymerase Chain Reaction (PCR) following the protocols of Silva-Oliveira et al. (2013), with an annealing temperature of 54ºC.

The primers set were based on sequences available for *E. coioides*, *E. akaara* (GU992890-EU399183) and anchored in intron 1, MHC_P0F (5'-TCAATACAGAGTTGGGCTG-3'), and in the region between exon 2 and intron 2, MHC_P2R (5'-AACGTTGTTCACACAGACCCTCTC-3'), which favored the isolation of the exon by the PCR technique. Posteriorly two new pairs of primers were designed to access the locus. The amplicons were then sequenced and specific primers were designed for the exon 2– MHC_FOR2 (5'-TTTGTTCCTCAGATGGATTTC-3') and MHC_REV (5'-TTGTTCACACAGACCCTCTCCTTCTC-3'). All the samples were sequenced using these new primers.

The sequences obtained were edited and corrected by Bioedit (Hall, 1999), being posteriously analysed in Mega 5 (Tamura et al. 2011). All the sequences were deposited in the GenBank (xxxxxxxxx).

The name of the locus (*MhcEit-DAB*) was defined based on the rules proposed by Ellis et al (2006). Exon 2 of the MHC IIβ chain was successfully amplified in 41 samples (13 homozygous), producing a sequence of 198 bp (excluding primers) and 66 aminoacids. The nucleotide sequences of the homozygous included 68 variable sites, 130 conserved sites, and eight singletons. The aminoacid sequences presented 34 conserved sites, 32 variable sites, and two singletons (Table 1). Stop codons were not detected in any of the sequences nor were multiple peaks observed in the chromatograms, both of which indicate that only a single locus was amplified.

Previous genetic data suggested that *E. itajara* grouper have low genetic diversity even at the most diversified mitochondrial genome region (Silva-Oliveira et al 2008) and the IUCN diagnosis indicates a significant population reduction in *E. itajara* (http://wwwiucnredlistorg/details/195409/0; accessed in 10/12/2013). Therefore, future studies including such marker may provide an important database for the understanding

of the evolutionary processes affecting *E. itajara* populations and the evaluation and modeling of their evolutionary fitness over the long term.

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Table Legends

Table 1 Alleles of the *MhcEit-DAB* locus identified in the *E. itajara* populations analyzed in the present study.

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Table

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