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Short Communication

Low levels of genetic diversity depicted from mitochondrial DNA sequences in a heavily exploited marine fish (*Cynoscion acoupa*, Sciaenidae) from the Northern coast of Brazil

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Abstract

The acoupa weakfish (*Cynoscion acoupa* - Sciaenidae) is a marine species of croaker with estuarine-dependent behavior, found in the western Atlantic from Panama to Argentina. It is one of the most exploited food fish on the northern coast of Brazil. In this study, DNA sequences were determined from the entire control region (D-loop) of the mitochondrial genome of 297 individuals collected during seven different months between December 2003 and August 2005 on the northern coast of Brazil (Amapá and Pará). Genetic variability expressed by haplotype (h = 0,892) and nucleotide (π = 0,003) diversities were low compared to other heavily exploited marine fish species from the western Atlantic and eastern Asia. AMOVA depicted a lack of genetic structuring among the samples from different years, indicating the presence of a single stock of *C. acoupa* within the sample area. The possible reasons for the low levels of genetic diversity are discussed. These results demonstrate a need for the monitoring of *C. acoupa* harvesting and the preservation of the estuaries within its geographic range, considering that this large fish depends on estuarine ecosystems during part of its life cycle.

Key words: Cynoscion acoupa, acoupa weakfish, Sciaenidae, low genetic diversity, mitochondrial DNA, D-loop.

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The Sciaenidae family encompasses 70 genera and 270 species of mainly marine and estuarine fish, distributed throughout the tropical areas of the Atlantic, Indian and Pacific oceans (Trewavas, 1977; Nelson, 1994). *Cynoscion*, one of the most important sciaenid genera in commercial terms, is represented by eight species on the Atlantic coast of South America (Chao, 1978). One of the most prominent local sciaenids is the acoupa weakfish, *Cynoscion acoupa*, which is known in Brazil as the *pescada amarela* or yellow fish, a demersal marine species dependent on estuarine ecosystems to complete its life cycle (Barletta-Bergan *et al.*, 2002). This species, which can reach a body length of 170 cm, is amply distributed between Panama and Argentina (Menezes and Figueiredo, 1980; Cervigón *et al.*, 1993), and is a very important fishery resource throughout its distribution. Fundação PROZEE (2006) reports that an average of almost 20 thousands of tons of *Cynoscion acoupa* were landed annually in the ports of Pará and Amapá between 2000 and 2005. The most productive year was 2000 (22.8 thousand tons), whereas catches declined by more than a third between 2003 and 2005 (21.8, 17.5 and 15.0 thousand tons, respectively), although the possible determinants of this pattern remain unclear.

Despite the commercial importance of *C. acoupa,* the population-level genetic variability of the species is unknown. In the present study, we used DNA sequences of the mitochondrial D-loop region to characterize the genetic diversity of the *C. acoupa* stock from northern Brazil - the Amazon coast - using samples collected over a three year period (2003 to 2005). We hope that the parameters provided by this screening will subsidize further genetic analyses and eventual management plans for the species at a regional level.

A total of 297 adult specimens of *C. acoupa* were obtained from the fish market at Bragança, in the Brazilian state of Pará, in December 2003 (n = 23), April (n = 14) and May (n = 400), 2004, and in April, June, July and August of 2005 (n = 19, 50, 41, and 108, respectively). Bragança is the third largest fishing port in Pará, and receives catches pri-

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marily from the fishing grounds of the states of Amapá and Pará, which straddle the Amazon estuary.

Total DNA was isolated from muscle tissue using the conventional phenol-chloroform protocol of Sambrook *et al.*, (1989). The following D-loop flanking primers were designed for this species: L1 5'-CCTAACTC CCAAAGCTAGGTATTC-3' and H2 5' CCGGCAGC TCTTAGCTTTAACTA - 3'. The Polymerase Chain Reaction (PCR) was carried out in a 25 µL reaction containing 4 µL of DNTP (1.25 mM), 2.5 µL of buffer (10X), 1 µL of $MgCl_2$ (50 mM), 0.25 µL of each primer (200 ng/µL), 1-2 µL of total DNA (50-100 ng/µL), 0.25 µL of Taq DNA Polymerase, *Invitrogen*, USA (5U/µL), and sterile water to complete the final volume. The reactions were performed using the following schedule: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C at 30 s - annealing at 57 °C per 1 min - extension at 72 °C for 2 min, and an final extension cycle at 72 °C for 7 min. The PCR products were purified with ExoSAP-IT (Amersham Pharmacia Biotech, USA) and submitted to the sequencing reaction using the Big Dye kit. Sequences were run in the ABI 377 (Applied Biosystems, USA), and deposited in GenBank under accession numbers EU562302-EU562598.

The D-loop sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) implemented in BIOEDIT (Hall, 1999). An unrooted neighbor-joining tree was obtained in PAUP (Swofford, 2003) using the Tamura-Nei algorithm (Tamura and Nei, 1993) as suggested by Modeltest 3.7 (Posada and Crandal, 1998). Bootstrap support for internal nodes was calculated using 1,000 replicates. Haplotype (h) and nucleotide (π) diversities were estimated according to Nei (1987), using DNAsp 4.1 (Rozas *et al.*, 2003). The variation among populations in different years was assessed using Analysis of Molecular Variance, AMOVA (Excoffier *et al.*, 1992), implemented in ARLEQUIN 3.1 (Excoffier *et al.*, 2005). The demographic history of *C. acoupa* was inferred using mismatch distribution analyses implemented in DNASP (Rozas *et al.*, 2003). The distribution is usually multimodal in samples drawn from populations at demographic equilibrium, but it is usually unimodal in populations following a recent demographic expansion (Rogers and Harpending, 1992). Mismatch distribution analyses, under the assumption of selective neutrality, were also used to evaluate possible historical events of population growth and decline (Rogers and Harpending, 1992). Theoretical distributions under the assumption of constant population size and the sudden expansion model were compared to the observed data. The goodness-of-fit of the observed data to a simulated model of expansion was tested with the sum of squared deviations and the raggedness index (Harpending, 1994), Tajima's *D* test (Tajima, 1989) and Fu's *F*s (Fu, 1997) tests. These tests were compared to the distribution expected under the neutral model as generated by 1,000 simulated re-samplings. The null hypothesis of neutrality may be rejected when a population has experienced demographic expansion, bottlenecking or heterogeneous mutation rate (Tajima, 1996).

The alignment generated in the present study encompasses 831 base pairs. Variation along this D-loop fragment was very low, however, with only 42 variable sites. Of the variable sites, 28 presented two variants (informative for parsimony) and 14 were singletons. Interestingly, no variable site was observed with three or more variants, indicating that most of the D-loop variability in *C. acoupa* is relatively recent. Overall, 83 different haplotypes were identified (Table 1). Fifty-five (66.26%) of these occurred just once, eleven were shared by only two individuals, whereas the remaining 14 haplotypes were found in three or more individuals. Haplotypes 1, 2, 3 and 12 were the only ones observed in all three years.

Moderate haplotype diversity (h) and very low nucleotide diversity (π) was observed in each year, and in the sample as a whole (Table 2). The AMOVA analysis indicated that there was no variation derived from differences among years and thus, no longitudinal population structuring. The phylogenetic tree for the whole population (2003-2005) depicted the 83 haplotypes in an unresolved topology with no statistical support for any of the internal branches - a typical star-like topology (Figure 1). The same random distribution pattern was obtained for each of the years sampled (not shown).

Sequences of the same segment of the mitochondrial D-loop have been employed in a number of studies to investigate genetic structuring and demographic history in populations of overexploited fishes, in which the observed values of h and π were much higher than those recorded for *C. ac*oupa in the present study. Seyoum *et al.*'s (2000) study of the sciaenid *Sciaenops ocellatus* from the Gulf of Mexico and Atlantic coast of the United States returned haplotype diversity of 0.98 and nucleotide diversity of 0.030, and the analysis of genetic structure indicated the existence of at least two distinct populations within the study area. Higher diversity values were also recorded in three lutjanid species: *Lutjanus campechanus* from the Gulf of Mexico and Florida (Garber *et al.*, 2004) with h = 0.97, π = 0.018, *Lutjanus purpureus* from Brazil (Gomes *et al.*, 2008) at h = 0.99 and π = 0.027, and *Lutjanus erythropterus* from eastern Asia (Zhang *et al.*, 2006), with values of h = 0.99 and π = 0.030. *Colossoma macropomum* (the tambaqui), one of the most heavily exploited freshwater fish in the Amazon basin (Santos *et al.*, 2007) returned values of h = 0.99 and π = 0.012. In the present study of *C. acoupa*, estimates of haplotype diversity were ten to fifteen percent lower than those recorded in these studies, while nucleotide diversity was three to ten times lower (Table 2). In common with these studies, but to a greater degree, most

Table 1 - Haplotype (Hap)frequencies in *Cynoscion acoupa*.

Hap	Sequences	Number
Hap1	CCGTCCACCCCTTAGTCCATATTTGCGTACGGTAAAATACGC	7
Hap2	..A..T.....	48
Hap3	.....T.....	78
Hap4	.....A.....	2
Hap5	.....T.....A...A.....	1
Hap6	.....T.....G.....	3
Hap7	...C.TG.....T.....	1
Hap8	.....T...T.....	1
Hap9	.TA..T..T..C.....T..G.....A.....	2
Hap10	.....T....A........C.........T.....	1
Hap11	.....T.........C.....	3
Hap12	.....T....A.....	3
Hap13	.....T.....T.....	1
Hap14	..A..T.....C.....	2
Hap15	..A..T........A..........A.....	2
Hap16	.....T.....C.....	2
Hap17	.....T........A.............A.....	2
Hap18	..A..T........A.....	2
Hap19	.....T.....A.....	5
Hap20	..A..T.....G.....	1
Hap21	..A..T.....A......C....	5
Hap22	..A..T........A....C.....	1
Hap23	..AC.T.....	7
Hap24	..A..T.....A.....A.	1
Hap25	.TA..T..T..C......G.G.....	2
Hap26	.....T.....G.....T..	2
Hap27	.TA..T..T..C......G.G.....A.....	3
Hap28	...C.TG...A.....	1
Hap29	..A..T.....C.....	1
Hap30	..A..T.....A.	1
Hap31	..A..T.....C.....G.....	1
Hap32	.....T.....A.....	3
Hap33	.....T.....G.....	1
Hap34	..A..T..........T......A.....	3
Hap35	...C.TG.....	5
Hap36	..A..T.....A.....	1
Hap37	.TA..T..T..C.....T..G.....A........G.....	1
Hap38	..A..T.....C.....	1
Hap39	..A..TG.....	3
Hap40	..A..T..T.....	3
Hap41	.....T........A..........A.....A.	1
Hap42	.TA..T.....	1
Hap43	..A..T.T.....	1
Hap44	.....T.....G.....A....A.....	1
Hap45	.TA..T..T..C........G.....	1
Hap46	..A..T.....G.G.....	1
Hap47	......G.......A.....A.....	1
Hap48	.....T.....C..C.....	2
Hap49	.....TG...A..G.....A.....	1
Hap50	......G.......A.....	1
Hap51	..AC.T........A.....	1
Hap52	.T.....	1
Hap53	..A..T.......G.....A.....	1
Hap54	..AC.T.....A.....	1
Hap55	..A.TT.....A.	1
Hap56	..A..T.....T.....A.....	1
Hap57	..A..T.....C.....	3
Hap58	T....T.....C.G.....	1
Hap59	..AC.....	1
Hap60	.....T.....T.....A.....	1
Hap61	..A..T.T.....A.....	1
Hap62	.....T....A.....T.....	1
Hap63	..A..T........A.....G...	1
Hap64	.....T.....A	1
Hap65	..A..T.T.....G.....	1
Hap66	.....T.....T.....	1
Hap67	..A..T.......G.....	1
Hap68	.....TG.....AG.....	1
Hap69	..AC.TG.....A.....	1
Hap70	.....T........A..T.....	1
Hap71	.....T........A......G.........A.....	1
Hap72	..A..T.....A.....	1
Hap73	......G.......A........C.....	1
Hap74	.....T.T......A.....	1
Hap75	..A..T........A.....A.....	1
Hap76	.....T........A...G.....	2
Hap77	..A..T.....C.....	1
Hap78	.....T....A.....C.....	1
Hap79	.TA..T..T..C......G.G.....A...A.....	1
Hap80	.....T.....C.....	1
Hap81	.....T.....C.G.....	1
Hap82	..A..T.....A.....	1
Hap83	.....T........A.....G.....	1

of the D-loop haplotypes observed in Brazilian *C. acoupa* are unique and recent (singletons).

Indices of neutral evolution (Tajima's *D* and Fu's *Fs*), applied to identify evidence of strong selective sweeps or balancing selection, were both negative and significant in all subpopulations as well in the population as a whole (D ≈ -1.8, p = 0.01 - Table 2). Fu's *Fs* statistic, which was devised specifically to detect population expansion and is more sensitive to the presence of singletons (as in the present case), was also highly significant in at least two of the three subpopulations (Table 1; *Fs* = -4.507 to -26.23). In addition, population expansion is indicated by the mismatch distributions (Figure 2), the low raggedness index, the star-like shape of the phylogenetic tree, and the ample distribution of the most common haplotypes. The raggedness index and SSD, and expansion parameters theta and tau estimated under the expansion model are presented in Table 2.

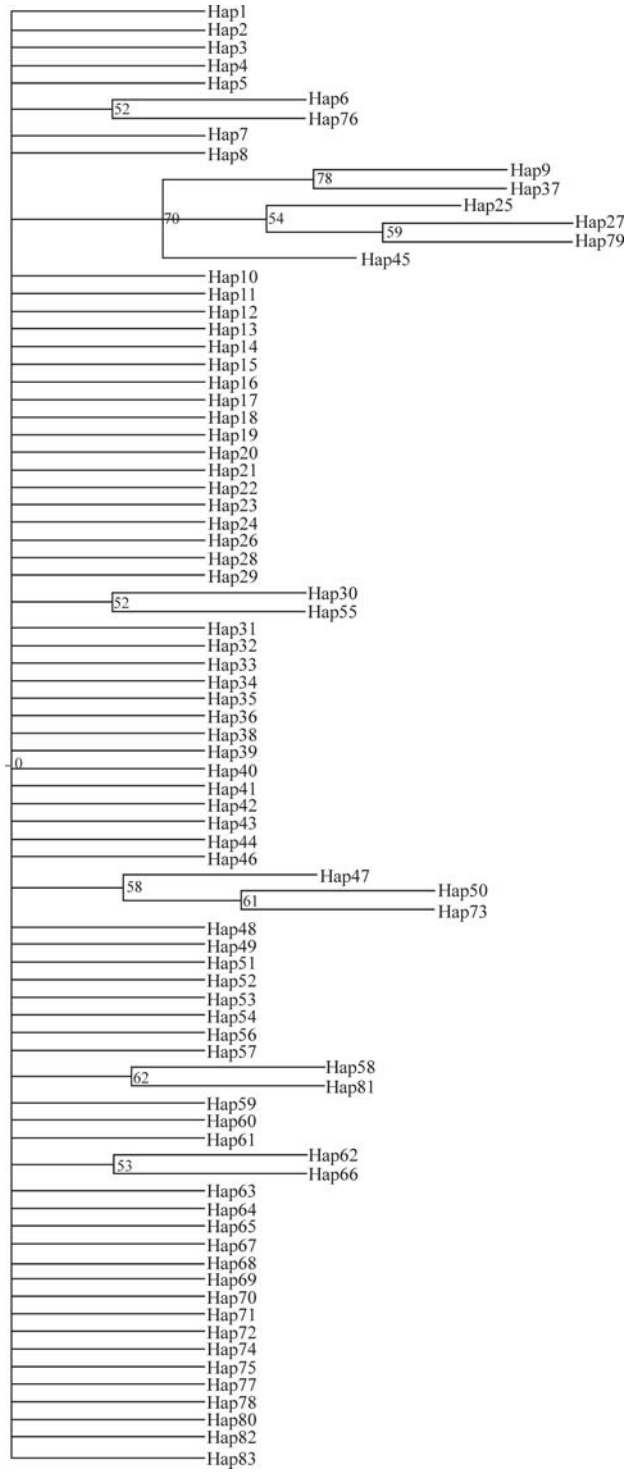

Figure 1 - Neighbor-joining tree of mitochondrial D-loop haplotypes in *Cynoscnion acoupa* from the northern coast of Brazil. Numbers at nodes indicate the percentage bootstrap support from 1,000 replicates (values of less than 50% are not shown).

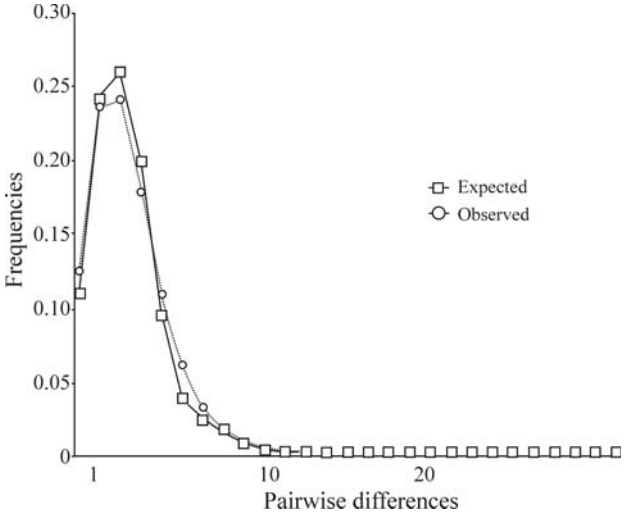

Figure 2 - Mismatch distribution for D-loop haplotypes in *Cynoscion acoupa* from the northern coast of Brazil. The expected frequency is based on a population growth-decline model (initial theta = 0.96, final theta = 1000, tau = 1.427), determined using the DNASP v4.20.2 program (Rozas *et al.*, 1993).

From our overall results, it seems clear that there is a single population of acoupa weakfish on the northern coast of Brazil, with no evidence of genetic structuring. This is an especially interesting result considering that the area from which the samples were collected straddles the Amazon estuary, potentially a major freshwater barrier. A single stock for this area implies that the Amazon plume is not blocking gene flow between subpopulations of *C. acoupa* from Amapá (to the north) and Pará (to the south).

However, it is not easy to identify the factor or factors responsible for the low levels of genetic diversity found in the *C. acoupa* stock. Overexploitation could be one factor because, despite recent improvements in technology, catches have declined progressively over the past few years. Overexploitation is identified by Dulvy *et al* (2003) as one of the two main causes of extinction of marine species, together with environmental degradation. These authors list more than 50 species of fishes for which overexploitation has been identified as the main cause of dramatic reductions in stocks in recent years. Nevertheless, only a few studies have related overexploitation conclusively with low levels of genetic diversity in fish populations. These include the New Zealand snapper *Pagrus auratus* (Hauser *et al.*, 2002), the North Sea cod *Gadus morhua* (Hutchinson *et al.*, 2003) and the dark blotched rockfish *Sebastes crameri* (Gomez-Uchida and Banks, 2006). While drastic reductions in stocks have been reported in a number of the species compared here with *C. acoupa*, such as the tambaqui (*Colossoma macropomum*), northern red snapper (*Lutjanus campechanus*) and red drum (*Sciaenops ocellatus*), no concomitant reduction in mtDNA diversity was observed.

If overexploitation is not the main cause of low levels of genetic diversity in *C. acoupa*, Dulvy *et al.* (2003) study would point to habitat degradation, although this seems unlikely in the present case, given that the Amazon coastline is still relatively sparsely populated and undeveloped. What may be more likely, considering the large number of single-

Table 2 - Parameters of D-loop diversity, mismatch analyses and mutation neutrality tests for each year in *Cynoscion acoupa* from the northern coast of Brazil.

Year	N1	H	π	*h*	SSD	Rg	Theta0	Tau	*Fs*	*D*
2003	25	12	0.00271	0.873	0.00867	0.04149	0.00176	2.78711	-4.507702*	-1.89049*
2004	54	26	0.00292	0.906	0.01186	0.05286	0.0000	2.79883	-19.64201**	-1.75991*
2005	218	70	0.00291	0.894	0.01081	0.05274	0.0000	3.03516	-26.23168**	-1.75926*
Total	297	83	0.00288	0.892	0.00091	0.03594	0.38496	1.83789	-16.62155*	-1.82357*

[1]N = number of specimens; H = number of haplotypes; π = nucleotide diversity; *h* = haplotype diversity; SSD = sum of squared deviation; Rg = Raggedness index; Theta and Tau = expansion parameters estimated under the expansion model; *Fs* = Fu's *Fs*; *D* = Tagima's *D*; * = $p < 0.05$; ** = $p < 0.01$.

tons identified in the present study, the star-like phylogenetic tree, and the evidence of rapid population expansion, is that *C. acoupa* has passed through a major bottleneck, which has erased much of its original variability, followed by a recent process of expansion. A similar explanation has been offered for the population mtDNA variability pattern observed in *Lutjanus campechanus* (Garber *et al.*, 2004) and *Lutjanus erythropterus* (Zhang *et al.*, 2006).

In conclusion, the present analysis has revealed that the *C. acoupa* population sampled from northern Brazil represents a single stock that occupies at least 1260 km of coastline (Amapá = 698 km, Pará = 562 km). This area encompasses a number of estuaries other than the Amazon, which may be important for the reproductive cycle of the species, and demand attention with regard to its conservation. In addition, the low levels of genetic variability observed here may compromise the evolutionary plasticity of this *C. acoupa* population. These findings indicate an urgent need for the careful monitoring of the harvesting of *C. acoupa* in northern Brazil.

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