MITOCHONDRIAL DNA POPULATION STRUCTURE IN WHITE MOUTH CROAKER (MICROPOGONIAS FURNIERI) ALONG THE ATLANTIC COAST OF BRAZIL

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Abstract

Variation in mitochondrial DNA (mtDNA) was examined among 149 white mouth croakers (*Micropogonias furnieri*) from six geographic locations in the Atlantic coast of Brazil (1° S – 34° S). Restriction fragment length polymorphism (RFLP) analysis of D-loop region detected a total of five composite haplotypes. Heterogeneity tests revealed no evidence of geographic differentiation in mtDNA haplotype frequencies within the region between 23° S and 34° S ($P = 0.263$). However, significant heterogeneity occurred between northern and southern region of 23° S ($P < 0.003$). Average sequence divergence revealed genetic differences in white croaker of the northern versus southern region ($\rho = 0.593\%$, $p < 0.05$). Analysis of molecular variance (AMOVA) indicated relatively high gene flow ($N_em = 3\text{-}44$) among south-central localities, but restricted gene flow ($N_em = 1\text{-}2$) between northern and southern regions. Collectively, these data are consistent with 1) a single stock of *M. furnieri* within the southern Brazilian coast and 2) separate, weakly differentiated stocks in the northern and the southern Brazil.

Introduction

The white mouth croaker (*Micropogonias furnieri*) is a euryhaline sublittoral sciaenid fish that uses estuarine and coastal waters as nursery and feeding grounds for larvae and juveniles (Isaac, 1988). *M. furnieri* supports an important demersal fishery along the Southwestern Atlantic. The species is distributed from the Yucatan Peninsula, Gulf of Mexico, at 20° N, to the Gulf of San Matias, Argentina, at 39° S (Chao, 1978), but it is particularly abundant on the southeastern shelf of Brazil (south of 23° S) and the shelf of Uruguay (Figure 1a). Recently, concern has been expressed over the apparent decline in the white mouth croaker fishery along the coast of Brazil, Uruguay and Argentina (Haimovici, 1998).

A thorough understanding of white mouth croaker population structure is essential for effective management of the fishery along the South Atlantic Ocean. The status of fish populations north of 23° S has not yet been investigated and the available information on the population structure south of 23° S appears contradictory. Variation in life history patterns and population dynamics suggest the existence of a population in the region between 23° S and 29°S (population I) and another between 29° S and 33°S (population II) (Vazzoler, 1991). Similar studies suggest that fishes inhabiting Rio de la Plata region probably form a third population (population III) on the coastal waters of Uruguay and that those further south form another (population IV) on the coast of Argentina (for review see Isaac, 1988). Populations II and III off southwestern Atlantic migrate southward (35° S) during summer and northward (27-28° S) during winter according to the seasonal displacements of the subtropical convergence system (Figure 1b). Previous genetic studies of *M. furnieri* using starch-gel electrophoresis of allozymes do not support the distinct-stock hypothesis. A high degree of genetic homogeneity in allele frequencies and also high levels of gene flow were found among white mouth croaker sampled from regions between 23° S and 40° S, and confirmed the hypothesis of a single population unit along the southwestern Atlantic coast (Maggioni *et al.* 1994; Levy *et al.*, 1998).

Recent studies have shown that restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) is useful in differentiating populations within several economically important fish species that exhibit little protein variation (Avise, 1994). The purposes of this paper were (1) to document the magnitude and spatial distribution of mtDNA variation in *M. furnieri* along the Brazilian coast, (2) to test further the hypothesis that white croaker

populations are subdivided, and (3) to estimate levels of genetic distance and gene flow among geographic localities.

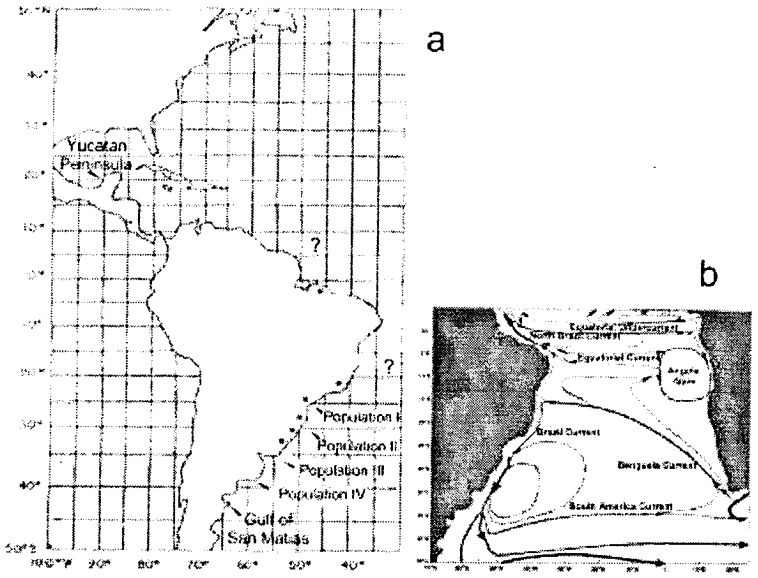

Figure 1. Distribution of *M. furnieri* on the Atlantic Ocean. (a) Distribution of assumed white croaker populations on the southwestern Atlantic. Spots indicate collection sites in the coast of Brazil. (b) Distribution of water currents on the South Atlantic Ocean (modified from Stramma and England, 1999).

Materials and Methods

Micropogonias furnieri of Brazilian coastal areas were obtained from commercial fishery during late 1999 and early 2000. Freshly caught tissue samples were removed and preserved at 95% ethanol. Dates and locations of capture and the size composition of the collections are presented in Table 1. Sample localities represent the northern (N), southeastern (SE) and southern (S) portions of the species Brazilian coast range, and also northern and southern regions of the hypothesized population boundary. Representative collections of

M. furnieri along the Northeastern (NE) coast were scarce.

Table 1. White mouth croaker *Micropogonias furnieri* collection data.

Sample	Location	Date	n	Standard length Mean, range (mm)
CH	33° 41' S 53° 27' W	3/2000	16	150, 130–200
RG	32° 00' S 52° 20' W	12/1999	32	320, 285–330
SC	29° 20' S 49° 43' W	12/1999	25	325, 295–360
SPR	25° 31' S 48° 30' W	1/2000	37	315, 290–340
RJ	22° 54' S 43° 13' W	2/2000	28	315, 285–325
PA	01°03' S 46° 46' W	4/2000	11	310, 285–320

Genetic population structure in *M. furnieri* was examined by using polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis (RFLP) of mitochondrial DNA (mtDNA). The studies were conducted at the "Laboratório de Bioquímica Marinha (LBM) Fundação Universidade Federal do Rio Grande (FURG)" in Rio Grande, RS. Mitochondrial DNA was obtained by the isolation procedure of Chow and Inowe (1993). Primers L-THR and 12 SAR-H were used in the polymerase chain reaction to amplify a 1450 bp product containing the entire D-loop region, tRNA-Pro and tRNA-Phe genes, and portions of the 12S rRNA and tRNA-Thr genes (Lankford Jr. *et al.*, 1999). Amplifications were performed in 50 µl reaction volume containing 1X PCR Buffer, 0.2 mM of dNTP, 2.0 mM of $MgCl_2$, 30 pmol of each primer, 1.5 U of *Taq* polymerase, and 20 ng of DNA template. PCR reactions were programmed for 35 cycles at 94° C for 1 min, 65° C for 1 min and 72° C for 2 min, including a final extension at 72° C for 8 min in the last cycle. PCR products were digested with the following nine restriction endonucleases: BglI, EcoRI, EcoRV, HaeIII, Hinf I, HpaII, PstI, RsaI, and SacII. Variant RFLP patterns were separated by gel electrophoresis on 1.5 % agarose gels and visualized after ethidium bromide with UV light illumination. Fragment sizes were estimated by using 1 Kb DNA ladder and Ø 174 – Hae III molecular weight markers, and UVIdoc software program, ver. 98.01. Distinctive restriction fragment patterns were identified by

letter codes and combined to produce composite mtDNA haplotypes for each individual fish.

Statistical analyses were performed with the Arlequin Package, ver. 2000 (Schneider *et al.*, 2000). The gene (nucleon) diversity and nucleotide diversity were calculated for each sample and for the pooled samples, according to Nei (1987). Percent mean nucleotide sequence divergences within and among white croaker samples were estimated by the average number of pairwise differences within and between populations (Nei and Li, 1979; Nei, 1987). Phenetic analysis of sample nucleotide sequence divergence matrix was carried out using UPGMA clustering (Sneath and Sokal, 1973). The distribution of haplotype frequencies was evaluated for homogeneity between samples using P-exact test of population differentiation (Raymond and Rousset, 1995) and a total of 10,000 steps in Markov chain. P-values < 0.003 were considered as significantly different, after Bonferroni sequential procedure. Population structure in *M. furnieri* was also calculated by using a hierarquical analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992). Samples were stratified by locality (PA, RJ, SPR, RSC, RG, and CH) and nested within region (N, SE, and S). Total genetic variation was partitioned into "within geographic localities", "among geographic localities", and "between regions". Significance of these components was tested using 1,000 random permutations. An Euclidian distance matrix between pairs of haplotypes was used for the calculation of F_{ST} values as an approximation of F-statistic (Weir and Cockerham, 1984). Gene flow (N_em) among localities was estimated as $F_{ST} = 2N_em + 1$. A matrix correlation (Mantel test) was carried out between the sample genetic distance matrix and a matrix of geographic distances (in miles) between all pairs of samples.

Results and Discussion

RFLP analysis of the D-loop regions of M. furnieri collected in the Brazilian coast revealed a total of five composite haplotypes (Table 2). Only two of the nine enzymes employed (HinfI and RsaI) produced variant patterns. The digestion profiles of variants were consistent with the hypothesis of single gains or losses of restriction sites.

Table 2. Distribution of *Micropogonias furnieri* mtDNA haplotypes based on restriction endonucleases among different collections. The order of restriction enzyme morphs, represented from left to right, is HinfI and RsaI.

Haplotype	Sample						Total
	CH	RG	RSC	SPR	RJ	PA	
h1 (AA)	12	27	17	33	22	5	116
h2 (AB)	0	1	3	0	0	0	4
h3 (AC)	4	2	1	3	1	0	11
h4 (BA)	0	2	3	1	5	6	17
h5 (BB)	0	0	1	0	0	0	1
Total	16	32	25	37	28	11	149

Of the 149 individuals surveyed, 116 (78%) shared the same composite mtDNA haplotype. The common haplotype (h1) was numerically dominant (> 0.70% frequency) at all localities except northern sample (PA, 0.45% frequency). The haplotype 4 occurred at 0.55% frequency in PA, at low (< 0.18%) frequencies in the other samples, and it was absent in CH sample. The haplotype 3 was present at 25% frequency in CH, at very low (< 0.08%) frequencies in the other samples, and it was absent in PA. The haplotype 2 occurred at low (< 0.12%) frequencies in RG and RSC samples, and the haplotype 5 at 0,04% frequency only in RSC sample.

Nucleon diversity averaged 0.388 ± 0.097 (mean ± SE) for the pooled sample, and ranged from 0.203 ± 0.084 in SPR to 0.546 ± 0.072 in PA. Nucleotide diversity also varied geographically, ranging from π= 0.086 ± 0.071 in SPR to π= 0.216 ± 0.141 in SC. Estimates of mtDNA nucleon and nucleotide sequence diversities indicated that genetic variation in M. furnieri is amongst the lowest than those reported in other marine fish species (Gold and Richardson, 1991, Graves et al., 1992, Lankford Jr. et al. 1999).

Percent nucleotide sequence divergences within and among samples are shown in Table 3. Average nucleotide divergence within (1.362%) and among (0.248%) pooled samples indicated that most of the observed mtDNA variation occurred within geographic localities. AMOVA also revealed that the majority (87.24%) of mtDNA variation in M. furnieri occurred within sample localities ($p<0.002$), but a significant portion (3.72%) was attributable to differences among localities ($p=0.047$). Variation between regions (9.04%) was unstructured ($p=0.112$).

Table 3. Average number of pairwise differences within population (ρ_{iX}, diagonal elements) and between populations (ρ_{iXY}, above diagonal); corrected average pairwise difference (below diagonal) (Nei and Li, 1979). Bolded elements were estimated as significantly different ($p < 0.05$).

	CH	RG	RSC	SPR	RJ	PA
CH	1.600	1.375	1.880	1.243	1.607	2.636
RG	0.066	1.018	1.496	0.876	1.121	1.963
RSC	0.107	0.014	1.947	1.422	1.593	2.233
SPR	0.056	-0.021	0.061	0.775	1.032	1.953
RJ	0.208	0.012	0.020	0.045	1.198	1.731
PA	1.018	0.636	0.441	0.748	0.313	1.636

The distribution and relative mtDNA haplotype frequencies support the general conclusions that white croaker population is weakly subdivided, with semi-isolated populations occurring in the north and south region of 23° S. Heterogeneity tests revealed significant differences in haplotype frequencies between PA and SPR samples, and between PA and CH samples ($P < 0.003$), which represent both limits of the total geographic species' range in this study (1° S – 34° S). Genetic differentiation, as estimated by average nucleotide divergence, was detected between pooled northern and southern localities (ρ= 0.593%, $p < 0.05$). Cluster-analysis of mtDNA genetic distances indicated that white croakers from these regions were distinguishable from one another (Figure 2). AMOVA indicated high level of population subdivision ($\phi ST = 0.322$, $p< 0.003$) and restricted gene flow (Nm = 1-2 effective female migrants per generation) between pooled northern and southern region. The matrix correlation analysis (Mantel test) revealed a geographic component to the distribution of the white croaker mtDNA haplotypes (correlation value = 0.883, $p < 0.003$).

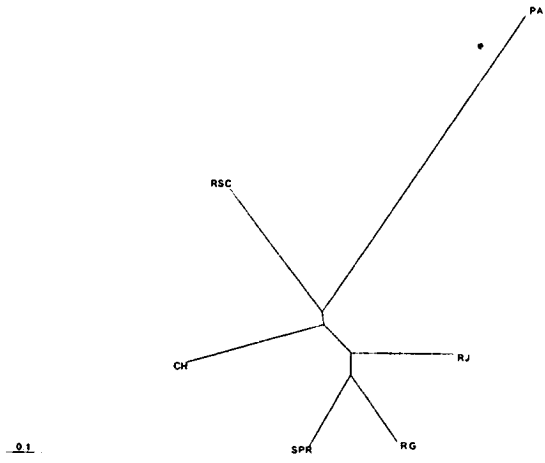

Figure 2. Phenetic tree showing the genetic distance (Nei and Li, 1979) among white mouth croaker sample localities.

The observed genetic heterogeneity between northern and southern Brazilian coast suggests that the number of migrants per generation is not sufficient to preclude genetic divergence of populations by random drift (Slatkin, 1987). The tropical and the subtropical circulation of different water masses within the South Atlantic Ocean may affect white croaker dispersal capability and restrict gene flow between regions. Stramma and England (1999) demonstrated that subtropical South Atlantic is governed by the subtropical gyre with a southward shift of the northern part of the gyre, while the tropical circulation shows several depth-dependent zonal current bands (Figure 1b). In the northern Brazil, the South Equatorial Current (SEC) in the near-surface layer reaches the shelf of Brazil (near 16° S) and separates into the southward flow of Brazil Current (BC) and at subsurface depth into the northward of the North Brazil Undercurrent (NBUC). Near the equator, the SEC overrides the subsurface of the NBUC and forms the surface intensified North Brazil Current (NBC). The southern Brazil is

influenced by the Subtropical Convergence system, made up of the confluence zone of southward flow of BC with the Falkland Current (FC).

There was, however, no genetic evidence of white croaker population subdivision within the southern Brazilian coast. Frequency- and distance- based analyses both suggested a single, panmictic population of white mouth croaker. The exact test of population differentiation revealed no heterogeneity in mtDNA haplotype frequencies among sample localities (P= 0.263). Low levels of mtDNA divergence among localities ρ= 0.011%), although not statistically significant, were more consistent with a pattern of semi-isolation by distance rather than marked subdivision by the oceanographic patterns of the subtropical convergence system. Low FST value (ϕST = 0.008) and high gene flow (Nm = 3-44 effective female migrants per generation) indicated a lack of geographic structure in the southeastern (23° S – 29°S) and southern (29° S – 34° S) regions. These results are consistent with allozyme data reported by Levy et al. (1998), suggesting that the number of migrants per generation among populations is high enough to maintain the homogeneity in gene frequencies and therefore, avoid differentiation by genetic drift. The lack of genetic heterogeneity found within the white croaker in both mtDNA and nuclear gene frequencies is supported by many other studies, despite the geographical variation of morphological and life history characters observed by Vazzoler (1991). Lima et al. (1996) showed that the general form of the circulation in the southern Brazilian shelf can be characterized by a combination of different processes that can restrict population subdivision and present high levels of gene flow in this area. There are, in fact, several aspects of white croaker biology and life history, which should facilitate dispersal and minimize geographic subdivision within south-central area of the Brazilian coast. White croakers are relatively strong swimmers, and adults form large schools offshore and are capable of extensive migration (Vazzoler, 1991). M. furnieri are long-lived pelagic spawners, meaning that individuals could spawn at multiple localities throughout their life-times (Isaac, 1988). Combined with these observations, the present mtDNA analysis supports that species dispersal capability results from interaction between physical environment conditions and species ecological requirements, and that life history variation when present, has an ecophenotypic basis.

At the level of genetic resolution employed in our study, we cannot disprove the null hypothesis that M. furnieri share a common gene pool. RFLP technique proved sufficiently powerful to detect white croaker population structure in the northern versus southern region of 23° S, but did not reveal structured genetic stocks of white croaker within the southern-central area of the Brazilian coast. The integrity of the hypothesized genetic stock boundary could be tested further by using fine-scale markers such as microsatellites, or direct sequencing of the

mitochondrial D-loop region, to provide greater resolution of geographic structure than RFLP analysis.

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