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Research Article

Genetic differentiation of *Macrodon ancylodon* (Sciaenidae, Perciformes) populations in Atlantic coastal waters of South America as revealed by mtDNA analysis

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Abstract

The king weakfish (*pescada-gó* in Portuguese - *Macrodon ancylodon* (Sciaenidae), a demersal (bottom-feeding) species found in South America Atlantic coastal waters from the Gulf of Paria in Venezuela to Baia Blanca in Argentina, is an economically important species because of its abundance and wide acceptance by consumers. Because of its wide distribution this fish may be subject to geographic isolation and this may have resulted in distinct populations along its coastal range. Considering that this species represents an important economic resource, confirmation of whether *M. ancylodon* is a single species or there are different genetic stocks spread over its wide distribution would be an important contribution to conservation policies and population management of the king weakfish. To investigate differences between king weakfish populations we used the cytochrome *b* and 16S rRNA genes to characterize *M. ancylodon* specimens caught throughout its South American range from Venezuela to Argentina. Our results clearly distinguished two genetically different groups which show nucleotide divergence and genetic structuring patterns that strongly suggest they may be different species, disagreeing with the widely accepted traditional taxonomy that accepts only one species of *Macrodon* in the western Atlantic.

Key words: Macrodon ancylodon, Sciaenidae, Perciformes, 16S rRNA, cytochrome *b*, genetic differentiation.

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Introduction

The family Sciaenidae contains about 70 genera and 270 species and is an important world fish resource (Nelson, 1984), this family being represented in South Atlantic waters by about 21 genera and 57 species, of which more than 30 are found along the Brazilian coast. A member of this family, *Macrodon ancylodon* Bloch and Schneider, 1801 (The king weakfish, *pescada-gó* in Portuguese) is a demersal (bottom-feeding) marine species found in South American Atlantic coastal waters from the Gulf of Paria in Venezuela to Baia Blanca in Argentina and is economically important because of its abundance and wide consumer acceptance (Cervigón, 1993; Haimovici *et al.,* 1996; Isaac and Braga, 1999).

Because of its wide distribution, *M. ancylodon* may be subject to geographic isolation with the consequent formation of distinct populations. In a study of the meristic and morphometric traits of *M. ancylodon* caught in southeastern and southern coastal waters of Brazil between latitudes 18°36' S and 32°10' S, Yamaguti (1979) distinguished four *M. ancylodon* populations with different mosaic patterns and spawning periods and restricted migration patterns, and suggested that there might be low gene flow between the populations and that geographic differentiation in the area studied might be caused by distinct environmental characteristics such as temperature and salinity. However, despite these meristic and morphometric findings genetic differentiation between *M. ancylodon* populations has not yet been investigated.

According to Brandini *et al.* (1997) differences in coastal geomorphology and oceanography influence the environmental characteristics of coastal waters as well as the composition, spatial distribution and temporal dynamics of the marine organisms which occur in such areas. Atlantic coastal waters of South America have distinct geomorphology and oceanographic features, with the distribution of coastal fishes in the western Atlantic being influenced by the discharge of large rivers and oceanic currents such as the North Brazil (Guyana), Brazil and Falklands currents as well as resurgence zones (Castro and Miranda, 1998; Palacio, 1982, Cervigón *et al*., 1993). In marine environments the geographic structure of populations may be influenced by local environmental conditions and the life history of the species, hence the potential for

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species dispersal does not always predict the amount of gene flow between geographically separated populations (Burton, 1983; Palumbi, 1995). Several factors are known to influence the dispersion of marine species (Palumbi, 1995), including spawning behavior, sharp temperature changes, salinity gradients and larval retention mechanisms (Sinclair, 1988). The geographical structure of a species is due not only to present gene flow but, more importantly, to historical gene flow between geographically separate populations (Slatkin, 1993).

Mitochondrial DNA has been extensively studied in fish and techniques based on mitochondrial DNA successfully applied to taxonomic questions, the well characterized cytochrome *b* gene having been particularly useful for the analysis of the relationship between recently diverged taxa such as populations and species (Stepien and Kocher, 1997) as well as being one of the genes most studied in fish systematics and Perciformes phylogeography (Roe *et al*., 1997; Briolay *et al.*, 1998; Durand *et al.,* 1999; Allergruci *et al*., 1999; Akihito *et al*., 2000; Farias *et al*., 2001; Streelman *et al*., 2002; Galbo *et al*., 2002). The 16S rRNA mitochondrial gene has also been shown to be a good marker to differentiate fish species, and has been used in comparative intergeneric and interspecific studies in several families of Perciformes (Ritchie *et al.,* 1997; Tringali *et al.,* 1999; Bernardi *et al.,* 2000; Hanel and Sturmbauer, 2000; Farias *et al.,* 2000; Craig *et al.,* 2001; Streelman *et al.,* 2002).

For any species, the success of conservation programs and the creation of effective management policies depends on determining the levels of genetic divergence within and between populations and developing strategies to maintain genetic diversity. Because *M. ancylodon* is an important economic resource, confirmation of whether or not it is a single species in the western Atlantic or whether different genetic stocks occur in over its wide distribution would represent a great contribution to conservation policies and population management. In the work reported in this paper, we used cytochrome *b* and 16S rRNA genes to characterize *M. ancylodon* populations throughout the geographic distribution of this species in South American coastal waters. The results clearly show two genetically different groups which have nucleotide divergence levels and genetic structuring patterns that suggest they may be different species, disagreeing with the traditional taxonomic system which allocates only one species to the genus *Macrodon* in the western Atlantic.

Materials and Methods

Sampling

A total of 69 *Macrodon ancylodon* Bloch and Schneider 1801 specimens were collected from the northern extreme of its range off the coast of Venezuela (five specimens), the coastal waters of eight Brazilian states (Amapá, Pará, Maranhão, Pernambuco, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul) (59 specimens) and from its southern limit off the coast of Argentina (five specimens) (Figure 1). The fish were identified according to the descriptions of Menezes and Figueiredo (1980) and Cervigón *et al.* (1993) and muscle tissue fragments collected and conserved in absolute alcohol or frozen in a freezer until DNA extraction.

DNA extraction, amplification and sequencing

Total DNA was isolated by the standard ribonuclease/proteinase/phenol/chloroform extraction method, followed by precipitation by sodium acetate/isopropanol (Sambrook *et al.,* 1989). The polymerase chain reaction (Mullis and Faloona, 1987) was used to amplify ≈500 base pair (bp) fragments of mitochondrial 16S ribosomal (16Sr) RNA and ≈800 bp fragments of the cytochrome *b* gene, Table 1 showing the primers and amplification conditions. Each 100 µL reaction mixture contained 16 µL DNTP (5 mM), 10 µL buffer solution (10x), 8 µL $MgCl_2$ (25 mM), 1 µL of each primer (200 ng/µL), 3-5 µL of sample DNA, 1U Taq DNA polymerase and sufficient purified water to complete the final volume (All reagents from Gibco BRL, USA). The PCR product was purified using the ExoSAP-IT enzyme (Amersham Pharmacia Biotech Inc., UK.) and sequenced by the chain-termination method (Sanger *et al*., 1977) using the ABI Prism™ Dye Terminator Cycle Sequencing Reading Reaction 'Big Dye kit' (Applied Biosystems, USA) following the manufacturer's instructions, the sequencing primers being the same as those used for the PCR. An inter-

Figure 1 - Collection locations for *Macrodon ancylodon* of the western Atlantic ocean.

Table 1 - PCR conditions for isolating cytochrome *b* and 16S rRNA genes of *Macrodon ancylodon.*

Gene	Flanking oligonucleotides	Amplification profiles*
Cyt *b*	L14725L: 5' CGAAACTAATGACTTGAAAAACCACCGTTG 3' HMVZ16: 5' AAATAGGAARTATCAYTCTGGTTTRAT 3'	30 cycles: 1 min at 94 °C (denaturation), 45 s at 40 °C (annealing), 90 s at 72 °C (extension)
rRNA16S	16 S1L: 5' CGCCTGTTTATCAAAAACAT 3' 16 S2H: 5' TTTCCCCGCGGTCGCCCC 3'	25 cycles: 1 mi at 94 °C (denaturation) , 1 min at 40 °C (annealing), 90 s at 72 °C (extension)

*For both programs we performed one step of initial denaturation at 94 °C for 3 min, and one step of final extension of 10 min at 72 °C.

nal 5'-CATTGGAGTAGTACTCTTCC-3' primer (GoCitbint, designed by I. Sampaio (pers. comm.)) was used to complete the 810 bp cytochrome *b* sequence. Sequencing was performed in an automatic ABI 377 sequencer (Perkin Elmer) using a fast 3.5 h run at 2400 V. The nucleotide sequence data determined for the present paper was deposited in GenBank under accession numbers AY253536-AY253656.

Phylogenetic analyses

Sequences were aligned by eye and edited in the XESEE program (Eyeball Sequence Editor, Cabot and Beckenbach, 1989). A *Paretroplus polyactis* (Cichlidae) cytochrome *b* sequence (Genbank AF370628) and a *Cynoscion arenarius* (Sciaenidae) 16S rRNA sequence (AF081679) were used as outgroups in the phylogenetic analyses which was accomplished using the molecular evolutionary genetics analysis (MEGA) version 2.0 (Kumar *et al.,* 2001) and phylogenetic analysis using parsimony (PAUP*) version 4.0 (Swofford, 2002) programs, the data analysis in molecular biology and evolution (DAMBE) program (Xia and Xie, 2001) being used to perform the saturation test and the MODELTEST program 3.06 version (Posada and Crandall, 1998) to choose the nucleotide substitution models suitable for this study. Estimation of the number of nucleotide substitutions was conducted for both genes using the method of Tamura and Nei (1993), the parameter of the gamma distribution (0.2752) being used only for the cytochrome *b* gene. Cladograms reflecting similarities between the populations were constructed using the distance matrix and maximum-parsimony techniques, deletions and insertions being treated as a fifth base in the parsimony analyses. The length of the trees (L), consistency index (CI), re-scaled consistency index (RC) and the homoplasy index (HI) were estimated for each case. The degree of confidence for the groups on the maximum-parsimony tree was evaluated by 1000 bootstrap replicates (Felsenstein, 1985).

Correlation between genetic distance and geographic distance

The Mantel clustering test, with 1000 permutations, was used to assess the correlation between the genetic distance and the geographic distance between populations (Mantel, 1967) using the Genetix program 4.02 version (Belkhir, 2001). Only the cytochrome *b* gene was used in this type of analysis, the genetic distance matrix being obtained using the method of Tamura and Nei (1993) and the geographic distance matrix constructed by transforming the latitude of the geographic coordinates of the sample points into kilometers, with the help of a program available on the site www.vsp.usp.br/grass/coop.html.

Results

Nucleotide composition

Table 2 shows that 29 different cytochrome *b* sequences were obtained from 53 *M. ancylodon* specimens, 766 of the 810 aligned sites being constant, 44 variable and 33 phylogenetically informative for parsimony. The nucleotide composition of the cytochrome *b* sequences was 23.6% adenine, 28.8% thymine, 32.8% cytosine and 14.8% guanine. A transition/transversion rate of 2.4 was observed in the first codon position, 1.6 in the second position and 3.0 in the third position, while the mean rate of transition/transversion substitution considering all the positions of the codon was 2.8.

Table 3 shows that 14 different 16S rRNA gene sequences were obtained from 68 *M. ancylodon* specimens, 14 of the 520 alignment sites being variable and 12 phylogenetically informative for parsimony. The mean base composition of this gene was 28.7% adenine, 23.6% thymine, 24.4% cytosine and 23.4% guanine, and the transition/transversion rate was 3.

Nucleotide divergence

Tables 4 and 5 present the nucleotide divergence matrices constructed using the method of Tamura and Nei (1993) for the two segments analyzed in the present study. The nucleotide divergence within each locality sampled was less than 1% for both genes. However, genetic distances between tropical (Venezuela to Pernambuco) and subtropical (São Paulo to Argentina) groups varied from 3.2% to 4.3% for the cytochrome *b* gene and from 2.0% to 2.4% for the 16S rRNA gene.

Figure 2 shows 24 nucleotide sites (45, 46, 55, 138, 174, 189, 198, 219, 234, 258, 270, 285, 321, 327, 372, 375, 414, 456, 457, 504, 558, 621, 627,726) in the cytochrome *b* gene that can be clearly distinguished in the tropical and subtropical *Macrodon* groups. Site 264 could also be considered a good marker, as all the individuals of the tropical

Table 2 - Specimens of *Macrodon* ancylodon whose cytochrome *b* was analyzed.

Origin	Specimens used in the analyses	Specimens sharing identical sequences
Venezuela	VE1	VE2, VE3, VE5
	VE4	-
Amapá	AP2	-
	AP6	-
	AP16	AP15, AP4, AP3
Pará	PA3	PA14
	PA4	-
	PA12	-
	PA16	-
Maranhão	MA2	MA3, MA4, MA5, MA6, MA9
	MA7	-
Pernambuco	PE3	PE9
	PE5	PE14
	PE7	-
São Paulo	SP3	-
	SP5	-
	SP7	-
	SP10	SP11
Paraná	PR1	PR5
	PR7	PR6, PR16
Santa Catarina	SC1	-
	SC2	-
	SC4	-
	SC5	SC6
Rio Grande do Sul	RS1	RS6, RS7
	RS2	-
	RS4	-
Argentina	ARG1	ARG2, ARG4, ARG5
	ARG3	-

group possess adenine, while the nucleotide sites (except for RS2 and ARG3) from the subtropical group possess guanine. Similarly, Figure 3 shows the 11 diagnostic sites (4, 19, 30, 35, 93, 112, 124, 244, 269, 281, 373) of the 16S rRNA gene which differentiate the two *Macrodon* groups.

Phylogenetic analyses

The two DNA segments studied did not show any indication of saturation in their sequences, so that both could be used in the phylogenetic analyses. Maximum-parsimony analyses considering all the taxa and the three codon positions of the cytochrome *b* gene resulted in six equally parsimonious trees (L = 193, CI = 0.964, RC = 0.945, HI = 0.036) and the consensus tree shown in Figure 4, while analysis of the 16S rRNA gene resulted in the single most-parsimonious tree (L = 50; CI = 0.98; RC = 0.964, HI = 0.02) shown in Figure 5. The maximum-parsimony and neighbor-joining trees for the two genes presented basically the same topology, showing two distinct clades (Figures 4 and 5), one clade included the specimens from the tropical group (Venezuela to Pernambuco) and the other included the populations from the subtropical group (São Paulo to Argentina). All the groupings were supported by significant bootstrap values greater than 93%.

Because of the low population genetic variability in the 16S rRNA and cytochrome *b* genes, clearly defined subgroups could not be identified in the tropical and subtropical groups either in the neighbor-joining or maximum-parsimony trees, so that that each group formed a highly polytomous clade.

Geographic distance versus genetic distance

The Mantel test used to assess the correlation between the genetic distances (of the cytochrome *b* gene) and geographic distances of the populations sampled resulted showed high correlation (r = 0.852), these results showing that there is a significant correlation (p) between geographic and genetic distances but only for the two main groups (tropical and subtropical) not for populations inside each group.

Discussion

Genetic characterization of *M. ancylodon*

From a commercial point of view, *M. ancylodon* is one of the most important Sciaenidae species found in South American Atlantic coastal waters, especially those off the northern and southern coasts of Brazil. Genetic char-

Table 3 - Specimens of *Macrodon* ancylodon whose 16S rRNA was analyzed.

Origin	Specimens used in the analyses	Specimens sharing identical sequences
Venezuela	VE1	VE2, VE3, VE4, VE5
Amapá	AP2	AP4, AP5, AP6, AP15, AP16
Pará	PA1	PA2, PA3, PA4, PA6, PA12, PA14, PA16
Maranhão	MA2	MA3, MA4, MA5, MA6, MA7, MA9
	MA8	-
Pernambuco	PE2	PE3, PE5, PE7, PE9, PE14
São Paulo	SP1	SP5, SP6, SP7, SP10, SP11
	SP2	-
	SP3	SP4
Paraná	PR1	PR4
	PR2	PR3, PR5, PR6, PR7, PR16
Santa Catarina	SC1	SC2, SC3, SC4, SC5, SC6
Rio Grande do Sul	RS1	RS2, RS3, RS4, RS5, RS6, RS7
Argentina	ARG1	ARG2, ARG3, ARG4, ARG5

Table 4 - Pairwise nucleotide divergence matrix estimated according to Tamura & Nei (1993) based on 810 base pairs of the cytochrome b gene for 29 sequences of *Macrodon*.

	Out	VE 1	VE 4	AP 2	AP 6	AP 16	PA 3	PA 4	PA 12	PA 16	MA 2	MA 7	PE 3	PE 5	PE 7	SP 3	SP 5	SP 7	SP 10	PR 1	PR 7	SC 1	SC 2	SC 4	SC 5	RS 1	RS 2	RS 4	ARG 1
Outgroup																													
Venezuela1	22.5																												
Venezuela4	23.0	0.2																											
Amapá2	22.8	0.4	0.6																										
Amapá6	22.8	0.4	0.6	0.2																									
Amapá16	22.5	0.0	0.2	0.4	0.4																								
Pará3	22.5	0.0	0.2	0.4	0.4	0.0																							
Pará4	22.7	0.4	0.6	0.2	0.2	0.4	0.4																						
Pará12	22.6	0.2	0.5	0.1	0.1	0.2	0.2	0.1																					
Pará16	22.7	0.1	0.4	0.5	0.5	0.1	0.1	0.5	0.4																				
Maranhão2	22.5	0.0	0.2	0.4	0.4	0.0	0.0	0.4	0.2	0.1																			
Maranhão7	22.6	0.2	0.5	0.1	0.1	0.2	0.2	0.1	0.0	0.4	0.2																		
Pernambuco3	22.5	0.1	0.4	0.5	0.5	0.1	0.1	0.5	0.4	0.2	0.1	0.4																	
Pernambuco5	22.2	0.4	0.6	0.7	0.7	0.4	0.4	0.7	0.6	0.5	0.4	0.6	0.2																
Pernambuco7	22.3	0.6	0.9	1.0	1.0	0.6	0.6	1.0	0.9	0.7	0.6	0.9	0.5	0.5															
São Paulo3	22.8	3.4	3.7	3.6	3.6	3.4	3.4	3.6	3.4	3.6	3.4	3.4	3.6	3.7	4.1														
São Paulo5	23.0	3.7	3.9	3.8	3.8	3.7	3.7	3.8	3.7	3.7	3.7	3.7	3.8	3.9	4.3	0.4													
São Paulo7	22.6	3.3	3.6	3.4	3.4	3.3	3.3	3.4	3.3	3.4	3.3	3.3	3.4	3.6	4.0	0.1	0.3												
São Paulo10	22.4	3.4	3.7	3.6	3.6	3.4	3.4	3.6	3.4	3.6	3.4	3.4	3.6	3.7	4.1	0.2	0.4	0.1											
Paraná1	22.6	3.3	3.6	3.4	3.4	3.3	3.3	3.4	3.3	3.4	3.3	3.3	3.4	3.6	4.0	0.1	0.3	0.0	0.1										
Paraná7	22.4	3.4	3.7	3.6	3.6	3.4	3.4	3.6	3.4	3.6	3.4	3.4	3.6	3.7	4.1	0.2	0.4	0.1	0.0	0.1									
Santa Catarina1	22.6	3.6	3.9	3.7	3.7	3.6	3.6	3.7	3.6	3.7	3.6	3.6	3.7	3.8	4.0	0.4	0.5	0.2	0.4	0.2	0.4								
Santa Catarina2	22.4	3.2	3.5	3.3	3.3	3.2	3.2	3.3	3.2	3.3	3.2	3.2	3.3	3.4	3.8	0.2	0.4	0.1	0.2	0.1	0.2	0.4							
Santa Catarina4	22.7	3.4	3.7	3.6	3.6	3.4	3.4	3.6	3.4	3.6	3.4	3.4	3.6	3.7	4.1	0.4	0.5	0.2	0.4	0.2	0.4	0.5	0.4						
Santa Catarina5	22.6	3.5	3.7	3.6	3.6	3.5	3.5	3.6	3.4	3.5	3.5	3.4	3.6	3.7	4.1	0.2	0.4	0.1	0.2	0.1	0.2	0.1	0.2	0.4					
Rio Grande do Sul1	22.6	3.4	3.7	3.6	3.6	3.4	3.4	3.6	3.4	3.6	3.4	3.4	3.6	3.7	4.1	0.2	0.4	0.1	0.2	0.1	0.2	0.1	0.2	0.4	0.0				
Rio Grande do Sul2	22.6	3.2	3.5	3.3	3.3	3.2	3.2	3.3	3.2	3.3	3.2	3.2	3.3	3.4	3.8	0.2	0.4	0.1	0.2	0.1	0.2	0.4	0.2	0.4	0.2	0.2			
Rio Grande do Sul4	22.8	3.4	3.7	3.6	3.6	3.4	3.4	3.6	3.4	3.6	3.4	3.4	3.6	3.7	4.1	0.4	0.5	0.2	0.4	0.2	0.4	0.5	0.4	0.0	0.4	0.4	0.4		
Argentina1	22.6	3.4	3.7	3.6	3.6	3.4	3.4	3.6	3.4	3.6	3.4	3.4	3.6	3.7	4.1	0.2	0.4	0.1	0.2	0.1	0.2	0.1	0.2	0.4	0.0	0.0	0.2	0.4	
Argentina3	22.9	3.5	3.8	3.6	3.6	3.5	3.5	3.6	3.5	3.6	3.5	3.5	3.6	3.7	4.1	0.5	0.5	0.4	0.5	0.4	0.5	0.6	0.5	0.6	0.5	0.5	0.3	0.6	0.5

Table 5 - Pairwise nucleotide divergence matrix estimated according to Tamura & Nei (1993) based on 520 base pairs of the 16S rRNA gene for 14 sequences of *Macrodon*.

	Out	VE	AP	PA	MA2	MA8	PE	SP1	SP2	SP3	PR1	PR2	SC	RS
Outgroup														
Venezuela	8.6													
Amapá	8.6	0.0												
Pará	8.6	0.0	0.0											
Maranhão2	8.6	0.0	0.0	0.0										
Maranhão8	8.8	0.2	0.2	0.2	0.2									
Pernambuco	8.6	0.0	0.0	0.0	0.0	0.2								
São Paulo1	8.6	2.2	2.2	2.2	2.2	2.4	2.2							
São Paulo2	8.3	2.4	2.4	2.4	2.4	2.6	2.4	0.2						
São Paulo3	8.3	2.0	2.0	2.0	2.0	2.2	2.0	0.2	0.4					
Paraná1	8.3	2.0	2.0	2.0	2.0	2.2	2.0	0.2	0.4	0				
Paraná2	8.6	2.2	2.2	2.2	2.2	2.4	2.2	0.0	0.2	0.2	0.2			
Santa Catarina	8.6	2.2	2.2	2.2	2.2	2.4	2.2	0.0	0.2	0.2	0.2	0.0		
Rio Grande do Sul	8.6	2.2	2.2	2.2	2.2	2.4	2.2	0.0	0.2	0.2	0.2	0.0	0.0	
Argentina	8.6	2.2	2.2	2.2	2.2	2.4	2.2	0.0	0.2	0.2	0.2	0.0	0.0	0.0

acterization of species of commercial interest is a primary condition for any fishing control policy, the study described in this paper being the first on the molecular characterization of the *M. ancylodon* populations throughout their geographic distribution. When the three cytochrome *b* codon positions were analyzed separately they differed greatly in base composition, the first position showing no bias but the second and third positions exhibiting a strong tendency towards decreased guanine content, this being especially true for the third codon where the guanine composition was only 5%. These values are similar to results obtained in several other groups of fish (Farias *et al.,* 2001; Durand *et al.,* 1999; Johns and Avise, 1998; Briolay *et al.,* 1998; Allergruci *et al.,* 1999; Roe *et al.,* 1997). Saturation was not observed in the 16S rRNA sequences.

Genetic differences in the *Macrodon* populations

The nucleotide divergence of the cytochrome *b* and 16S rRNA genes was very low at the interpopulational level (< 1%) and this was randomly distributed in the tropical and subtropical clades. Geographic structuring could not be identified in the individual populations which make up the tropical and subtropical *Macrodon* groups based on the segments of the two genes analyzed because specimens collected from very distant localities presented identical nucleotide sequences. Taking the genetic divergence results for the cytochrome *b* gene as an example, specimens from Venezuela (VE1), Amapá (AP16), Pará (PA3) and Maranhão (MA2) had sequences which were 100% similar (Table 4) and the results for the 16S rRNA gene were similar (Table 5), suggesting that there are no barriers preventing continuous genetic flow between the populations within each group. The low nucleotide divergence values for the two genes are often observed in closely related taxa, our results with the cytochrome *b* gene being similar to population studies on fish such as *Dicentrarchus labrax* (Allergruci *et al.,* 1999) and *Epinephelus marginatus* (Gilles *et al.,* 2000).

When we compared the nucleotide sequences of the tropical *Macrodon* group with the subtropical group we found, surprisingly, high nucleotide divergence, with values reaching 4.3% for the cytochrome *b* gene and 2.6% for the 16S rRNA gene. Twenty-five sites of the 810 bp of the cytochrome *b* gene and 11 of the 520 bp of the 16S rRNA gene are diagnostic in terms of separating the tropical and subtropical *Macrodon* groups (Figures 2 and 3), nucleotide divergence being of the same magnitude as the values detected for intrageneric comparisons in Perciformes. For the cytochrome *b* gene close-related species of *Selene* (*Selene setapinis*, *S. orstedi* and *S. dorsalis*, Carangidae) and *Scarus* (*Scarus coelestinus* and *S. guacamaia*, Scaridae) show nucleotide divergence values varying from 2% to 4% (Reed *et al*., 2001; Streelman *et al.*, 2002). Additionally, the 16S rRNA shows divergences varying from 1 to 4% between different Perciformes species of the same genus as observed in the centropomid *Centropomus* (Tringali *et al*., 1999), in the cichlids *Geophagus*, *Satanoperca*, *Retroclus* and *Aequidens* (Farias *et al.,* 2000), in the sparids *Dentex, Diplodus, Pagellus* and *Pagrus* (Hanel and Sturmbauer, 2000), in the pomacentrids *Stegastes, Pomacentrus, Chromis, Amphriprion* and *Abudefduf* (Tang, 2001), in the scarids *Chororus, Scarus* and *Sparisoma* (Bernardi *et al*., 2000; Streelman *et al*., 2002) and the serranids *Epinephelus* (Craig *et al.,* 2001). The level of variation detected by us between the tropical and subtropical *Macrodon* groups is

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          1111222222333344455667
       44537891356782277155052222
       56584989484051725467481776
PPO    CGGATCAATTCCTCCAAATACCCTA
VE1    .AAT..GTGAAT.T..GG.G.G.C.
VE4    .AAT..GTGAAT.T..GG.G.G.C.
AP2    .AAT..GTGAAT.T..GG.G.G.C.
AP6    .AAT..GTGAAT.T..GG.G.G.C.
AP16   .AAT..GTGAAT.T..GG.G.G.C.
PA3    .AAT..GTGAAT.T..GG.G.G.C.
PA4    .AAT..GTGAAT.T..GG.G.G.C.
PA12   .AAT..GTGAAT.T..GG.G.G.C.
PA16   .AAT..GTGAAT.T..GG.G.G.C.
MA2    .AAT..GTGAAT.T..GG.G.G.C.
MA7    .AAT..GTGAAT.T..GG.G.G.C.
PE3    .AAT..GTGAAT.T..GG.G.G.C.
PE5    .AAT...TGAAT.T..GG.G.G.C.
PE7    .AAT..GTGAAT.T..GG.G.G.C.
SP3    T..CCTC.AGG.C.TG..C.T.T.G
SP5    T..CCTC.AGG.C.TG..C.T.T.G
SP7    T..CCTC.AGG.C.TG..C.T.T.G
SP10   T..CCTC.AGG.C.TG..C.T T.G
PR1    T..CCTC.AGG.C.TG..C.T.T.G
PR7    T..CCTC.AGG.C.TG..C.T.T.G
SC1    T..CCTC.AGG.C.TG..C.T.T.G
SC2    T..C.TC.AGG.C.TG..C.T.T.G
SC4    T..CCTC.AGG.C.TG..C.T.T.T
SC5    T..CCTC.AGG.C.TG..C.T.T.G
RS1    T..CCTC.AGG.C.TG..C.T.T.G
RS2    T..CCTC.AGA.C.TG..C.T.T.G
RS4    T..CCTC AGG.C.TG..C.T.T.T
ARG1   T..CCTC.AGG.C.TG..C.T.T.G
ARG3   T..CCTC.AGA.C.TG..C.T.T.G
```

Figure 2 - Cytochrome *b* informative sites that discriminate tropical (Venezuela to Pernambuco) and subtropical (São Paulo to Argentina) *Macrodon* groups. The dots represent the same nucleotide as occurs in the first sequence for that site in the outgroup species *Paretroplus polyactis* (PPO). The codes on the left refer to the geographic location where the *Macrodon ancylodon* specimens were caught: VE, Venezuela; AP, Amapá; PA, Pará; MA, Maranhão; PE, Pernambuco; SP, São Paulo; PR, Paraná, SC; Santa Catarina; RS, Rio Grande do Sul; ARG, Argentina.

```
          112223
       1339124687
       49053244913
CAR    GCACAGG-CTA
VE1    A.TT..AGGC.
AP2    A.TT..AGGC.
PA1    A.TT..AGGC.
MA2    A.TT..AGGC.
PE2    A.TT..AGGC.
SP1    .A.-GA.AA.G
SP2    .A.-GA.AA.G
SP3    .A.-GA.AA.G
PR1    .A.-GA.AA.G
PR2    .A.-GA.AA.G
SC1    .A.-GA.AA.G
RS1    .A.-GA.AA.G
ARG1   .A.-GA.AA.G
```

Figure 3 - Mitochondrial 16S rRNA informative sites that discriminate tropical (Venezuela to Pernambuco) and subtropical (São Paulo to Argentina) *Macrodon* groups. . The dots represent the same nucleotide as occurs in the first sequence for that site in the outgroup species *Cynoscion arenarius* (CAR). Deletions are represented by dashes. The codes on the left refer to the geographic location where the *Macrodon ancylodon* specimens were caught: VE, Venezuela; AP, Amapá; PA, Pará; MA, Maranhão; PE, Pernambuco; SP, São Paulo; PR, Paraná, SC; Santa Catarina; RS, Rio Grande do Sul; ARG, Argentina.

strong evidence indicating that these two groups should not be considered as a single species.

Phylogenetic analyses

The neighbor-joining (NJ) and maximum-parsimony (MP) trees for the two genes had the same topology and gave two distinct clades, one for the tropical group and one for the subtropical group (Figures 4 and 5), all the trees being supported by highly significant bootstrap values (≥ 93%). There was no perfect geographic structuring of the sampled populations in the clades, as is shown by specimens from one locality grouping with specimens from another, geographically distant, locality. Gilles *et al.* (2000) used cytochrome *b* gene sequences to study *Epinephelus marginatus* (Serranidae) populations and observed that in spite of high nucleotide similarity between French and Tunisian populations there was a certain geographic structuring of the specimens and that cladograms showed separate French and Tunisian populations. Allergruci *et al.* (1999), studying *Dicentrarchus labrax*, also observed that in spite of the high level of nucleotide similarity in the cytochrome *b* gene sequences there was a certain geographic structuring within Mediterranean *D. labrax* populations, with populations from the northwest (French waters) and east (Greek and Egyptian waters) being clearly distinct from Tyrrenian populations. In our work, the lack of geographic structuring in the *Macrodon* populations within each group is indicative of recent genetic flow.

Our data corroborates the existence of two panmitic and genetically isolated *Macrodon* populations, one, the tropical group, being distributed from Venezuela to Pernambuco and the other, the subtropical group, occurring from São Paulo to Argentina.

Based on morphometric traits, Yamaguti (1979) distinguished four *M. ancylodon* populations in southeastern and southern Brazilian coastal waters but our results, based on specimens collected in the same regions, did not agree with these findings even when the rapidly evolving cytochrome *b* gene was considered, as can be seen in the MP and NJ trees shown in Figure 4.

Isolation of the *Macrodon* populations

According to Dawson *et al.* (2001) intra and interspecific diversity patterns are greatly influenced by historically established barriers to marine animal move-

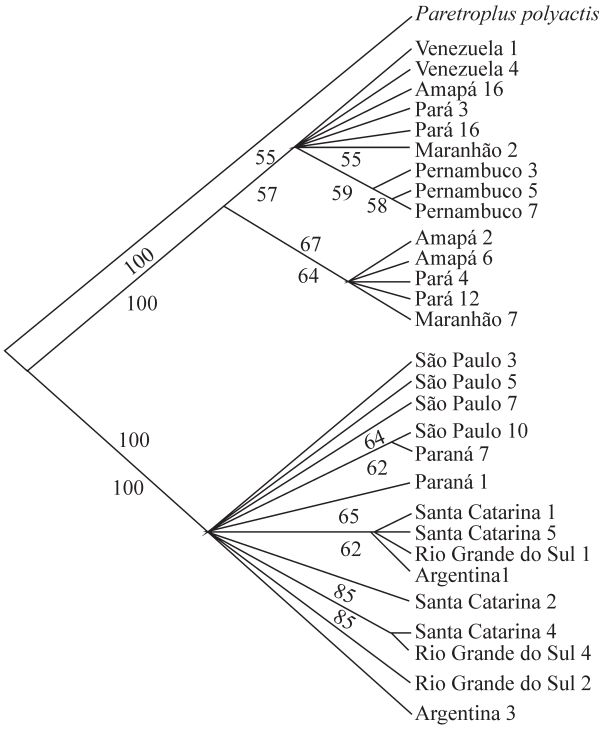

Figure 4 - Phylogenetic tree for *Macrodon* populations based on 810 base pairs of cytochrome *b*. When above 50%, numbers in the nodes indicate the percentage of trees out of 1000 bootstrap replicates for the maximum-parsimony (MP, above) and neighbor-joining (NJ, bellow) trees. *Paretropuls poplyactis* was used as outgroup.

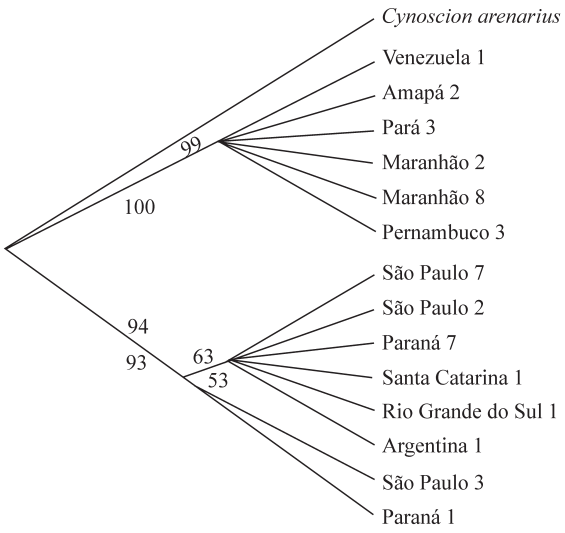

Figure 5 - Phylogenetic tree for *Macrodon* populations based on 520 base pairs of 16S rRNA. When above 50%, numbers in the nodes indicate the percentage of trees out of 1000 bootstrap replicates for the maximum-parsimony (MP, above) and neighbor-joining (NJ, bellow) trees. *Cynoscion arenarius* was used as outgroup.

ment created by circulation patterns, temperature regimens and coastal topography. In our study, we observed that there is marked geographic structuring in the genus *Macrodon* that diverges at the Brazilian northeast into two genetically distinct clades (tropical and subtropical). This pattern of separation between the groups fits the type I phylogeographic pattern proposed in the recent classification by Avise (2000) where the lines are defined by haplotype groups spatially separated by an effective barrier to genetic flow.

The Mantel test showed a positive correlation between genetic and geographic distances, although significant geographic structuring for *M. ancylodon* was detected only between, not within, the tropical and subtropical groups, indicating that factors other than isolation by distance could be contributing to differentiation in this species. A possible cause for the marked genetic differentiation of the tropical and subtropical *Macrodon* groups could be patterns of oceanic circulation caused by surface marine currents, *e.g.* the North Brazil and Brazil currents that diverge at the Brazilian northeast and influence different regions of the coast. The North Brazil current influences part of the northeastern and all the northern coast of Brazil as far as Venezuela, while the Brazil current influences part of the northeastern coast and the southeastern and southern coast of Brazil and some regions of the Argentinean coast (Castro and Miranda, 1998; Brandini *et al.,* 1997; Palacio, 1982). Our analyses showed that the two *Macrodon* groups consist of populations from areas that are influenced by different surface currents and therefore we can suggest that this is one of the barriers that prevents genetic flow and isolates the *Macrodon* populations of the tropical and subtropical groups.

Another factor that could be isolating the *Macrodon* groups is the difference in sea water temperature in the tropical and subtropical regions, temperatures ranging from 24 to 28 °C in the tropical regions (Castro and Miranda, 1998; Cervigón, 1993) while the subtropical waters are characterized by lower temperatures which range from 27 °C to 18 °C and can reach 16 °C in the Campos basin of the Brazilian state of Rio de Janeiro (Castro and Miranda, 1998). The *Macrodon* populations in the two groups probably developed mechanisms to adapt to the type of environment where they lived and this served as a barrier enhancing the geographical isolation of the populations. Indeed, Lowe-Mcconnell (1999) states that water temperature is one of the main factors that affect the distribution of marine fish, while Schroth *et al.* (2002) observed that water temperature is one of the main factors that affect distribution and genetic differentiation in medusa jelly fish (*Aurelia* sp.) and suggests that ecological data indicates that adaptation to local climatic conditions may have forced the diversification of this organism during its evolutionary history. Stepien (1995) has suggested that the barrier of warm water in the south of the Gulf of California is the cause of the isolation and consequent genetic divergence which occurs among spotted sand bass (*Paralabrax*

maculatofasicatus) populations in the Gulf of California and the Pacific coast.

According to some authors, population isolation can occur during strong alterations in environmental conditions, such as climatic changes and geological phenomena (Stepien *et al.,* 2001; Brunner *et al.,* 2001; Lundberg *et al.*, 1998). The ice ages are also considered one of the phenomena that caused changes in environmental conditions, resulting in isolation and later differentiation of populations (Beheregaray *et al.,* 2002; Brunner *et al.,* 2001). According to Yamaguti (1979), *M. ancylodon*, originally from a tropical region, arrived at the cooler southern Brazilian waters during the postglacial periods and by successive adaptive radiations became adapted to these new environments. The occupation of cooler water environments may have caused the development of adaptation mechanisms to these environments and later isolation of tropical and subtropical populations.

Another important factor affecting *Macrodon* isolation could be the difference in reproductive periods. Studies carried out in the tropical and subtropical regions of Brazil have shown that this species has distinct reproduction periods, December and February in the South (Juras and Yamagguti, 1989; Yamaguti, 1967; Vazzoler, 1963) and July-August and October-December in the north (Camargo-Zorro, 1999).

Macrodon ancylodon: a single species in the western Atlantic?

According to the allopatric speciation model, temporary geographic isolation is required to form reproductive barriers (Sturmbauer *et al.,* 1997). Futuyma (1997) postulated that low levels of genetic flow and population isolation are important for speciation to take place. Several studies have assessed the degree of genetic differentiation of fish populations based on mitochondrial DNA sequences, especially cytochrome *b* and D-loop region sequences (Dawson *et al.,* 2001; Gilles *et al.,* 2000; Allergruci *et al.,* 1999).

The low divergence values which we found in the *M. ancylodon* populations within the tropical and subtropical groups suggest high intra-group genetic flow. In the phylogenetic trees (Figures 4 and 5), the populations cannot be separated because individuals belonging to very distant geographical regions group together. Even when specimens from the same or close geographic regions compose a clade, the grouping is not statistically supported. Conversely, high genetic divergence was observed between the tropical and subtropical groups, and this is also confirmed by the separation of these groups in the phylogenetic trees with the nodes being supported by high bootstrap values (Figures 4 and 5). The levels of divergence found between tropical and subtropical *M. ancylodon* groups for the two segments of the mitochondrial genome studied are of the same magnitude, or greater than, those found for individual specimens of different species within the same genus. These results suggest that these two groups have already reached a stage of sufficient genetic differentiation to be considered as distinct species and not just as a single species for the western Atlantic, as is traditionally accepted in the current taxonomy based on morphological data. The evidence presented in this paper indicates that the taxonomic status of *Macrodon ancylodon* should be revised, and that strategies for management and conservation of stocks from different regions of the Brazilian coast should take in consideration the results of the present investigation.

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