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Lack of genetic differentiation in the fat snook *Centropomus parallelus* (Teleostei: Centropomidae) along the Brazilian coast

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The patterns of genetic variation in a fragment of the cytochrome *b* mtDNA gene in 130 individuals of the fat snook (*Centropomus parallelus*) did not show evidence of genetic differentiation along the Brazilian coast as shown in *F*-statistics and AMOVA analyses, indicating the existence of high gene flow among the studied populations. In addition, the study of the evolutionary demography of the species using mismatch distribution analysis and statistical tests such as Tajima's *D* and Fu's *F* indicate that it experienced a pattern of population expansion during its recent evolutionary history.

Key words: marine fish; mtDNA; phylogeography; west Atlantic.

The fat snook *Centropomus parallelus* is a tropical and sub-tropical marine–estuarine species widely distributed in the Atlantic Ocean from the state of Florida in southern U.S.A. to the state of Santa Catarina in southern Brazil (Rivas, 1986; Tringali *et al.*, 1999). It is found in coastal waters, estuaries and lagoons, making incursions into freshwater and occasionally into hypersaline lagoons (Rivas, 1986). *C. parallelus* is an important species for recreational and commercial fisheries and has aquaculture potential (Alves *et al.*, 2006; Lemos *et al.*, 2006), yet no study to date has investigated how the genetic variability of this species is distributed throughout its range. Such knowledge is fundamental for designing management strategies that ensure the long-term conservation of the target species and the sustainability of its fisheries (Laikre *et al.*, 2005).

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MtDNA has been widely used as an efficient marker in population genetics studies and fisheries management, including a variety of marine fish species (Seyoum *et al.*, 2000; Garber *et al.*, 2004, 2005). In addition to provide an assessment of the degree of gene flow and geographical structure among different regions, mtDNA can be used to infer the evolutionary demography of a species from the distribution of pair-wise sequence differences in a sample (mismatch distribution) (Rogers & Harpending, 1992).

The goal of the present study is two-fold. First, the population genetics and geographical structure of *C. parallelus* in different locations of the Brazilian coast are characterized using a fragment of mtDNA. Second, the demographic history of *C. parallelus* was inferred using mismatch distribution analysis.

Samples of *C. parallelus* were obtained from six locations along the Brazilian coast: Santa Catarina (26°27′ S; 48°36′ W, $n = 18$), Paraná (25°41′ S; 48°38′ W, $n = 70$), Espírito Santo (19°23′ S; 40°04′ W, $n = 13$), Bahia (13°00′ S; 38°30′ W, $n = 11$), Sergipe (11°05′ S; 36°56′ W, $n = 11$) and Maranhão (2°23′ S; 44°02′ W, $n = 7$), from December 2005 to December 2006. Small pieces of tissue (gills or muscle) were preserved in ethylenediamine tetra-acetic acid – dimethylsulphoxide (EDTA–DMSO) buffer (Seutin *et al.*, 1991) and stored at −20° C until being processed for molecular analysis.

Genomic DNA was extracted using the EZ-DNA kit (Biosystems, Sao Paulo, Brazil) according to the manufacturer's instructions. The primers for the mitochondrial gene cytochrome *b* (cyt *b*) (GluDG.L 5′-TGACCTGAARAACCAYCGTTG-3′ and H16460 5′-CGAYCTTCGGATTACAAGACCG-3′) were used for the amplification of a *c.* 1400 bp fragment of which only the middle 769 bp were used in the analysis (where the obtained electropherograms were most reliable).

Polymerase chain reaction (PCR) amplifications were performed in 25 μl reactions containing 4 ng μl^{-1} of template DNA, 1× buffer, 3·4 mM $MgCl_2$, 0·6 mM of dNTP, 0·2 μg μl^{-1} of bovine serum albumin, 0·12 U of Taq DNA polymerase, 3 μM of each primer. Cycling conditions included an initial denaturation at 94° C for 4 min, followed by 40 cycles of 94° C for 45 s, 56° C for 45 s and 72° C for 30 s and a final extension at 72° C for 10 min. PCR products were electrophoresed in a 1·5% agarose gel, stained with ethidium bromide and visualized under UV light. Successfully amplified products were purified using MinElute™ spin columns (Qiagen, Valencia, CA, U.S.A.) or Microcon® Centrifugal Filter Units (Millipore, Billerica, MA, U.S.A.). DNA concentrations and purity were measured using a spectrophotometer (GeneQuant Pro; Amersham Biosciences, Little Chalfont, U.K.).

Sequencing reactions were carried out in 10 μl solutions including the following final concentrations: 5 ng μl^{-1} of template DNA, 0·5 μl of Big Dye (Applied Biosystems, Foster City, CA, U.S.A.), 0·16 μM of each primer and 0·1× of reaction buffer. The final product was purified using Sephadex G50 or ethanol–isopropanol precipitation and processed on an ABI3130 automatic sequencer (Applied Biosystems).

Forward and reverse strands were reconciled using STADEN 1.6.0 (Staden, 1996). Sequences contained no indels. The degree of genetic differentiation among the studied populations was assessed using traditional *F*-statistics.

The population demographic history in *C. parallelus* was inferred using the mismatch distribution analysis (Rogers & Harpending, 1992; Slatkin & Hudson, 1991). Three parameters are estimated using Rogers & Harpending's (1992) model: $\theta_0 = 2N_0u$, $\theta_1 = 2N_1u$ and $\tau = 2ut$, where an initial female population of effective size N_0 is assumed to grow rapidly to a new size of N_1 at a time t generations before the present, and u is the per-generation probability that a mutation strikes a particular nucleotide in the region under study. These parameters were estimated using the generalized non-linear least-square approach developed by Schneider & Excoffier (1999). Also, the degree of approximation between the observed mismatch distribution and the expected under population growth was tested using Harpending's (1994) raggedness statistic. Departures from neutrality were tested using the statistics D (Tajima, 1989) and F_s (Fu, 1997). The significance of D and F_s was tested by randomization. D and F_s are calculated for each simulated data set to obtain an empirical null distribution of these statistics and hence the probability of the observed D and F_s under the hypothesis of drift-mutation equilibrium. All analyses were carried out as implemented in ARLEQUIN 3.1 (Excoffier *et al.*, 2005).

Sequences from of 130 individuals of *C. parallelus* from six locations off the Brazilian coast (Santa Catarina, Paraná, Espírito Santo, Bahia, Sergipe and Maranhão) were included in the analysis [GenBank accession numbers EU927346–EU927364; Fig. 1(a)]. Average nucleotide composition was as follows: C, 31·3%; T, 29·3%; A, 25·9% and G, 13·3%. Variation in the studied regions included 18 transitions and one transversion. Samples exhibited relatively low nucleotide diversity (π), with values ranging from 0·001 to 0·003

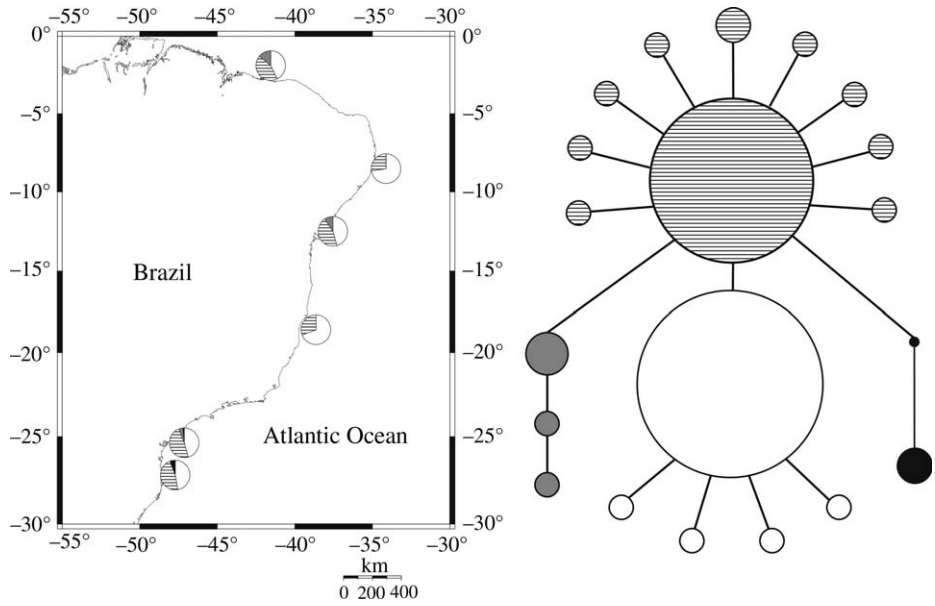

FIG. 1. Sampling locations for *Centropomus parallelus* along the Brazilian coast and their respective haplotype relationships. Pie charts in each location indicate the relative proportion of each clade at that site.

TABLE I. Levels of genetic diversity and estimated parameters from mismatch distribution analysis of *Centropomus parallelus* at six locations off the Brazilian coast

	All samples	Santa Catarina	Paraná	Espírito Santo	Bahia	Sergipe	Maranhão
Sample size	130	18	70	13	11	11	7
Number of haplotypes	19	5	12	2	4	4	2
Tajima's D	−1·06	−1·06	−1·79	−0·13	−0·74	−1·03	0·21
P_D	<0·001	0·18	0·01	0·42	0·28	0·19	0·67
Fu's F_S		−17·87	−1·35	−8·12	−0·17	0·76	0·11
P_F		0·00	0·11	0·00	0·33	0·64	0·01
Raggedness index r	0·023	0·012	0·013	0·022	0·230	0·070	0·290
P_r	0·31	0·00	0·22	0·23	0·07	0·30	0·31
θ_0 (95% CI)	0·00 (0·00–0·70)	0·00 (0·00–0·19)	0·00 (0·00–0·19)	0·00 (0·00–0·31)	0·00 (0·00–0·33)	0·00 (0·00–0·33)	0·00 (0·00–0·4)
θ_1 (95% CI)	207 062 (3·88–4373)	∞ (2·14–∞)	∞ (2·74–∞)	2·93 (1·39–∞)	∞ (3·53–∞)	∞ (5·10–∞)	83 332 (2·51–∞)
τ (95% CI)	0·98 (0·35–1·24)	1·06 (0·14–2·14)	1·01 (0·41–1·58)	0·79 (0·00–2·16)	0·88 (0·00–2·00)	1·46 (0·00–3·48)	1·09 (0·00–2·52)

(Table I). Although 19 haplotypes were identified, two of them were present in >80% of the samples and were distributed throughout all sampled locations [Fig. 1(a)].

Pair-wise F_{ST} values between populations were non-significant and varied from low to slightly negative (Table II), indicating a high level of gene flow among the studied stocks of *C. parallelus*. In addition, no structure was detected when comparing southern samples (Paraná and Santa Catarina) against northern samples (Sergipe, Bahia and Espírito Santo) using an AMOVA (Table III), with 97% of the genetic variation occurring within rather than between groups of populations. Therefore, these results failed to show any evidence of genetic structuring among samples from six locations along the Brazilian coast, despite the substantial geographical distances among them.

Given that no evidence of geographical structure was detected among the studied populations, the mismatch distribution of samples of all location were combined into a single analysis and showed a unimodal pattern characteristic of population expansion (Fig. 2). Estimates of Tajima's *D* and Fu's *F* were negative in all locations, although the associated probabilities were not statistically significanct in some cases (Table I).

Centropomus parallelus did not show evidence of genetic differentiation among the studied populations, suggesting the existence of high gene flow. This is shown by the low observed F_{ST} values. Marine fish commonly have pelagic eggs, larvae, juveniles or adults free of physical geographic barriers, often leading to a continuous distribution of fish through the oceans (Avise *et al.*, 1987). However, a study of the congener *Centropomus undecimalis* (Bloch) in the Gulf of Mexico and Caribbean Sea using allozymes and RFLP data showed relatively high F_{ST} values, with the formation of at least two subpopulations (Tringali & Bert, 1996). Given the broad geographical distribution of *C. parallelus*, it is still possible that geographical differentiation is present elsewhere in its distribution, yet the broad sampling in the present study indicate that special conditions, such as the currents associated with the Gulf of Mexico, might be necessary to elicit such a pattern.

TABLE II. A description of molecular distances (upper diagonal, indicating mean percentage of pair-wise differences), geographical distances (upper diagonal, in parenthesis, measured in km), average number of pair-wise differences within each population (main diagonal) and average F_{ST} values (lower diagonal) of the studied populations. SC, Santa Catarina; PR, Paraná; ES, Espírito Santo; BA, Bahia; SE, Sergipe; MA, Maranhão. None of the estimates was statistically significant at $\alpha = 0{\cdot}05\%$

	SC	PR	ES	BA	SE	MA
SC	0·97	0·91 (100)	0·82 (1350)	0·99 (2050)	1·02 (3300)	0·86 (4100)
PR	−0·02	0·90	0·80 (1250)	0·96 (1950)	1·00 (2200)	0·82 (4000)
ES	−0·04	−0·03	0·62	0·87 (700)	0·76 (950)	0·75 (2750)
BA	0·05	0·06	0·03	1·09	1·07 (250)	0·88 (2050)
SE	0·03	0·04	0·02	−0·05	0·98	0·95 (1800)
MA	−0·06	−0·07	−0·11	0·03	0·03	0·86

TABLE III. Analysis of molecular variance (AMOVA) separating southern samples (Paraná and Santa Catarina) and northern samples (Sergipe, Bahia, and Espírito Santo). The estimated F_{ST} for this dataset is 0·03

Source of variation	Sum of squares	Variance components	Percentage variation
Among groups	1·04	0·014	3·05
Among population within groups	1·11	−0·005	−1·01
Within populations	53·23	0·451	97·97
Total	55·38	0·460	

Nucleotide diversity for the cyt *b* gene was surprisingly low (0·001–0·003) for *C. parallelus* from all sampled locations, particularly given the fairly large geographical distribution of the species. Higher levels of nucleotide diversity (between 0·021 and 0·053) were detected in other marine fish species using the mtDNA control region, such as *Xiphius gladius* L. (Rosel & Block, 1996), *Sciaenops ocellatus* L. (Seyoum *et al.*, 2000), *Lutjanus campechanus* (Poey) (Garber *et al.*, 2004) and *Acanthocybium solandri* (Cuvier) (Garber *et al.*, 2005). This difference can be attributed to the conservative nature of the cyt *b* gene, although other studies on the same gene in other species also showed higher nucleotide diversity (Bucklin *et al.*, 1997; Wood & Raley, 2000; Árnason, 2004).

The observed mismatch distributions and the negative Tajima's *D* and Fu's values observed for all locations in this study are consistent with a recent population expansion (Slatkin & Hudson, 1991; Rogers & Harpending, 1992). Similar results have been obtained in other marine fishes, such as *L. campechanus* (Garber *et al.*, 2004). Future studies using multilocus datasets can provide valuable information on the time frame of this expansion.

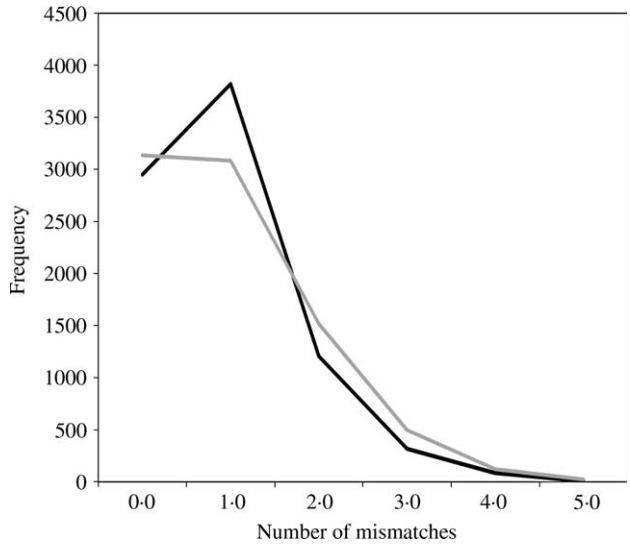

FIG. 2. Mismatch distribution of the studied cytochrome *b* sequences of *Centropomus parallelus* ($n = 130$ sequences) (—, observed; —, model).

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