



Population Structure of *Lutjanus purpureus* (Lutjanidae - Perciformes) on the Brazilian coast: further existence evidence of a single species of red snapper in the western Atlantic

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ABSTRACT

The present study focus on the mitochondrial control region to investigate phylogeographic patterns and population structure in *Lutjanus purpureus*, and to evaluate the genetic similarity between *L. purpureus* and *L. campechanus*. For the initial analysis, 810 base pairs sequence from control region were obtained from 239 specimens of *L. purpureus* collected from four localities off the Brazilian coast. The results revealed the presence of a single panmictic population characterized by high values of genetic diversity. The 299 base pairs hypervariable portion were used for the combined analysis of *L. purpureus* and *L. campechanus*, being 275 haplotypes identified in the 414 specimens. Phylogenetic tree and haplotype network did not indicate phylogeographic substructuring between the two species, but rather an intense intermingling of individuals. Considering their marked morphological similarity, the molecular data presented here indicate that only one species of red snapper exists in the western Atlantic.

Key words: control region, *Lutjanus purpureus*.

INTRODUCTION

The family Lutjanidae includes four subfamilies – Etelinae, Apsilinae, Paradichthyinae and Lutjaninae – that together encompass 107 species (Iwatsuki et al. 1993, Nelson 2006, Moura and Lindeman 2007), 20 of which are found in the western Atlantic (Allen 1985, Cervigón 1993). Most snapper species are commercially important fishery resources, exploited in a predatory fashion on either an industrial or an artisanal scale, as in the case of the red snapper, *Lutjanus purpureus* (Poey 1867), known in Brazil

as the *pargo* or *vermelho*. According to Cervigón (1993) and Allen (1985), this species is distributed throughout the Caribbean, south of Cuba, and Brazil - from northeast as far south as the state of Bahia. However, some authors such as Carpenter and Nelson (1971) and Rivas (1966) argue that the species can be found in some parts off the coast of the United States. The species is found primarily at depths of between 30 and 160 meters in areas with a sandy or rocky bottom (Paiva 1997), but is especially common at 70-120 m (Allen 1985). The adults are normally either solitary or found in small groups, whereas the juveniles congregate

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in large shoals (Szpilman 2000). Long-lived, but slow-growing (R.F.C. Souza, unpublished data) the largest individuals of this species may reach a total length of 100 cm, although most adult fish are around 65 cm (Allen 1985).

Off the northern and eastern coast of Brazil, the species is caught mainly on the continental shelf and oceanic reefs (Ivo and Hanson 1982). Industrial harvesting began in Brazil in the 60s, and concentrated on the oceanic reefs off the northeastern coast (Fonteles-Filho 1972). Subsequently, the focus shifted to the continental platform, from the state of Ceará westwards to the Amazon coast of the states of Amapá, Pará, and Maranhão (Ivo and Sousa 1988, Paiva 1997). In the present day, the species is harvested mainly off Amapá and Pará.

Given the implications of such processes for the maintenance of stocks, there is growing interest in the genetic structure of the populations of commercially important fish species (Garber et al. 2004, Palstra et al. 2007, Santa Brígida et al. 2007, Rodrigues et al. 2008, Silva-Oliveira et al. 2008). These and other studies have generated an essential database for the development of systematic management strategies for fish stocks. The data also permit the analysis of evolutionary processes and past demographic events (Frankham et al. 2004). Studies of this kind usually focus on the mitochondrial control region and microsatellite markers, highly variable repeated sequences found in the nuclear genome.

Very little is known about the population structure of the red snappers of the western Atlantic, especially regarding molecular markers. However, Salles et al. (2006) conducted a pioneering study using a molecular tool (the mitochondrial cytochrome b gene, together with morphological variables) for the analysis of the structure of the red snapper populations off the northern coast of Brazil. The results of this study indicated the presence of two distinct stocks of *L. purpureus* on the northern coast of Brazil associated with different fishery

zones. Four haplotypes were identified, two of which were associated with the western zone (47° - 49° W), coinciding with the mouth of the Amazon, and two with the eastern zone (43° - 46° W). A similar division was identified in the morphometric study of Sousa-Júnior et al. (2002), suggesting a fragmentation degree of the populations in this region, based on its vast area and environmental heterogeneity.

In their analysis of biological characteristics, oceanographic conditions, and the evolution of fishing practices, Ivo and Hanson (1982) formulated two hypotheses to define the Brazilian stocks of red snapper and their migratory patterns. Their first step was to divide the region into four zones, two of which are mentioned above. These zones (III and IV) are associated with the Amazon estuary, and characterized by an extensive continental shelf, and relatively low salinity levels. By contrast, zones I and II refer to the Caiçara and Ceará oceanic reefs, which are separated from the continental shelf by deep depressions. These areas are characterized by high salinity, and a predominance of calcareous algae on the ocean floor.

One hypothesis presented by Ivo and Hanson (1982) is that only a single stock of Brazilian red snappers exists. This stock is composed of individuals originating from distinct breeding seasons. The alternative to this would be the presence of two stocks, resulting from geographically distinct breeding populations. In both cases, the adults migrate between feeding areas on the continental shelf (zones III and IV), to breeding areas on the oceanic reefs (zones I and II). The eggs and larvae would be transported to nursery areas on the continental shelf by the Guianas current, the difference being that, in the case of the second hypothesis, each stock would migrate to breed at a different reef.

Recently, Gomes et al. (2008) analyzed northern Brazilian populations of *L. purpureus* based on sequences of the mitochondrial control region, and found evidence of intense gene flow between populations, indicating the presence of a

single genetic stock. Furthermore, comparisons with a second snapper species, *Lutjanus campechanus* revealed greater variation within than between populations, indicating a lack of phylogeographic structuring, and the probable existence of only a single species of red snapper in the western Atlantic, as suggested by Cervigón (1993). These findings are supported by the marked morphological similarities of the two forms (Rivas 1966, Allen 1985, Moran 1988, Cervigón 1993), as well as their many shared characteristics, such as their longevity, slow maturation, and reproductive patterns (Moran 1988, Wilson and Nieland 2001, Souza et al. 2003, R.F.C. Souza, unpublished data). The existence of a single red snapper species in the western Atlantic would also be consistent with the situation observed in other *Lutjanus* species (Cervigón 1993).

In general, taxonomic uncertainties arise from a lack of data for analyses. The adequate definition of species is a decisive step in the evaluation of conservation status, and the development of effective management strategies (Frankham et al. 2004). In the present study, the mitochondrial control region was analyzed in order to evaluate the genetic structure and phylogeographic attributes of Brazilian populations of *L. purpureus*. In addition to the expansion of the analysis of Gomes et al. (2008), in terms of the number and distribution of samples, a complementary analysis of the hypervariable portion of the control region included the sequences obtained from western Atlantic *L. campechanus* by Garber et al. (2004).

MATERIALS AND METHODS

SAMPLES

The *L. purpureus* specimens analyzed in the present study were collected at four areas on the Brazilian coast (Fig. 1), two in northern coast of Brazil, one in Ceará and one in Bahia, providing a representative sample of the Brazilian distribution of the species (Allen 1985, Cervigón 1993). The samples from northern coast

were obtained from local fishing ports, as well as the Northern Regional Center for Fishery Research and Development (CEPNOR from Portuguese), as part of collaboration with the Universidade Federal Rural da Amazonia (UFRA). The samples from Ceará and Bahia were obtained from fishing companies in the cities of Fortaleza and Salvador, respectively. Only specimens with a reliable known origin were included in the study. All procedures were carried out according to the international practices for animal use and care under the control of an internal committee of the Universidade Federal do Pará, Brazil.

Morphological identification of the specimens was carried out in the field, and then tissue samples (muscle and/or tongue) were extracted and taken to the Genetics and Molecular Biology Laboratory of the Bragança Campus of the Universidade Federal do Pará (UFPA), where they were stored in the Lutjanidae tissue bank.

In order to test the genetic differentiation hypothesis proposed by Sousa-Júnior et al. (2002) and Salles et al. (2006), the samples from northern coast of Brazil (CNB) were divided into two groups, CNB I and CNB II, according to a longitudinal gradient. Group I included all the specimens captured between longitudes 46° and 50° W, while Group II included the individuals taken between 43° W and 45° W. This subdivision follows the red snapper fishery zoning scheme of Ivo and Hanson (1982), in which "subarea IV" corresponds to CNB I, and "subarea III" to CNB II.

A total of 239 specimens of *L. purpureus* were collected for the present study, 119 of which from area CNB I, 49 from CNB II, 43 from Ceará, and 28 from Bahia. An additional 175 sequences of the congener *L. campechanus* were obtained from the literature (Garber et al. 2004). These specimens were collected in the western Atlantic and the Gulf of Mexico, and include six individuals from Alabama, 13 from Cancún (Mexico), six from Louisiana, 115 from Mississippi, and 35 from the Atlantic coast of Florida (Fig. 1).

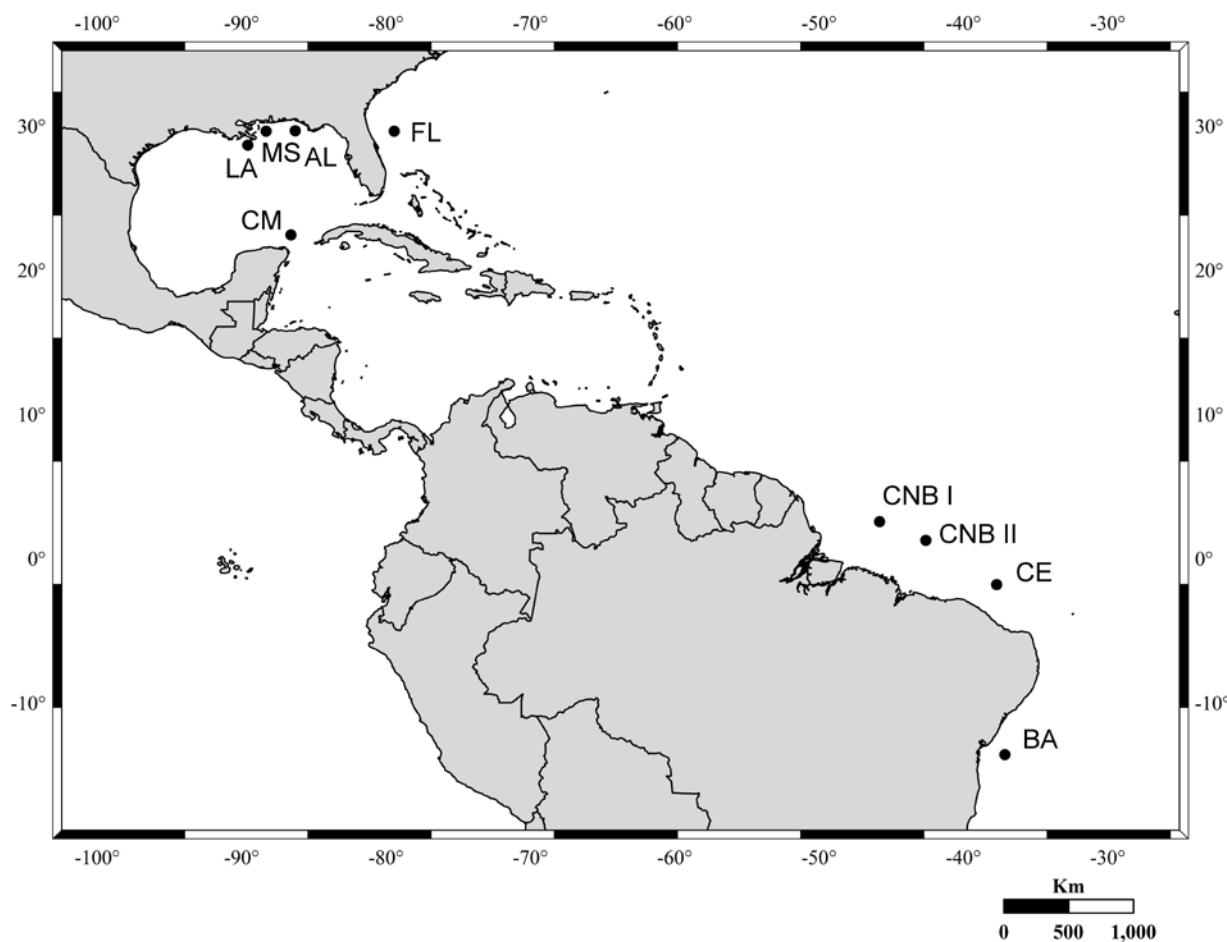


Fig. 1 - Locations of the areas in which the specimens of *L. purpureus* (Brazil) and *L. campechanus* (United States and Mexico; Garber et al. 2004) were collected. LA: Louisiana; MS: Mississippi; AL: Alabama; FL: Florida; CM: Cancun, México; CNB: northern coast of Brazil (CNB I – Longitudes: 46° and 50° W; CNB II – Longitudes: 43° and 45° W); CE: Ceará; BA: Bahia.

EXTRACTION OF DNA, AMPLIFICATION AND SEQUENCING OF THE MITOCHONDRIAL CONTROL REGION

Isolation of the genomic DNA was carried out using the technique developed by Sambrook and Russel (2001), which involves digesting enzymes (RNase, Proteinase K), phenol, chloroform, and isoamyl alcohol for the precipitation of the proteins, and alcohol (isopropanol and ethanol) for the precipitation of the DNA. The mitochondrial control region was isolated and amplified using the Polymerase Chain Reaction (PCR) technique, using the primers Dloop-A-5'-TTCCACCTCTAACTCCCAAAGCTAG-3' and Dloop-G-5'-CGTCGGATCCCATCTTCAGTGT

TATGCTT-3 (Lee et al. 1995), which are located on the transfer RNA (tRNAs) that flank the control region, the proline and phenylalanine tRNAs, respectively. Additionally, the primers Dloop-F-5'-ACTTTCATCGACGCTTGCA-3' and Dloop-R-5'GTGATCTTAGGAGTATAAGGG-3' were used to isolate the hypervariable region described by Garber et al. (2004), and thus permitting the analysis of practically the whole control region. Figure 2 shows the location of the hypervariable region sequenced by Garber et al. (2004), and the fragments sequenced by Gomes et al. (2008) and in the present study.

The PCRs were conducted in a final volume of 25 µL, which included: 4µL of the mixture of

deoxynucleotides (1.25mM), 2.5 µL of the *Taq* enzyme buffer (10 x), 0.75 µL of MgCl₂ (50mM), approximately 100 ng of the total DNA extracted from the sample, 0.25 µL of each primer (200 ng/µL), 0.2 µL of Taq DNA polymerase (5U/µL - Invitrogen) and sterile distilled water to complete the final reaction volume. The amplification protocol was as follows: initial denaturation for 3 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C (denaturation), 1 minute at 57°C (hybridization) and 2 minutes at 72°C (extension). At the end of the 35 cycles, there was a final extension of 7 minutes at 72°C.

The amplicons were purified using the ExoSAP-IT kit (Amersham Pharmacia Biotech Inc., UK) according to the maker's instructions. Once purified, the samples were sequenced using the dideoxyterminal method of Sanger et al. (1977) with a Big Dye reagent kit (ABI Prism™ Dye Terminator Cycle Sequencing Reading Reaction).

The precipitated product was sequenced in an ABI 3100 automatic capillary sequencer (Applied Biosystems – Perkin Elmer). The primers used for the sequencing reaction were Dloop-A (Lee et al. 1995) and Dloop-F (Garber et al. 2004).

SEQUENCE EDITION AND ALIGNMENT

The sequence files and the electropherograms were transferred to a computer for alignment and edition, using BioEdit (Hall 1999). Once the nucleotides were conferred, the sequences containing errors or uncertainties were corrected. An automatic multiple alignment was then performed in the Clustal-X application (Thompson et al. 1997), followed by a new visual inspection and correction of the insertions or deletions codification as necessary. Two databases were produced, the first covering the 810 bp of the mitochondrial control region identified in the Brazilian specimens of

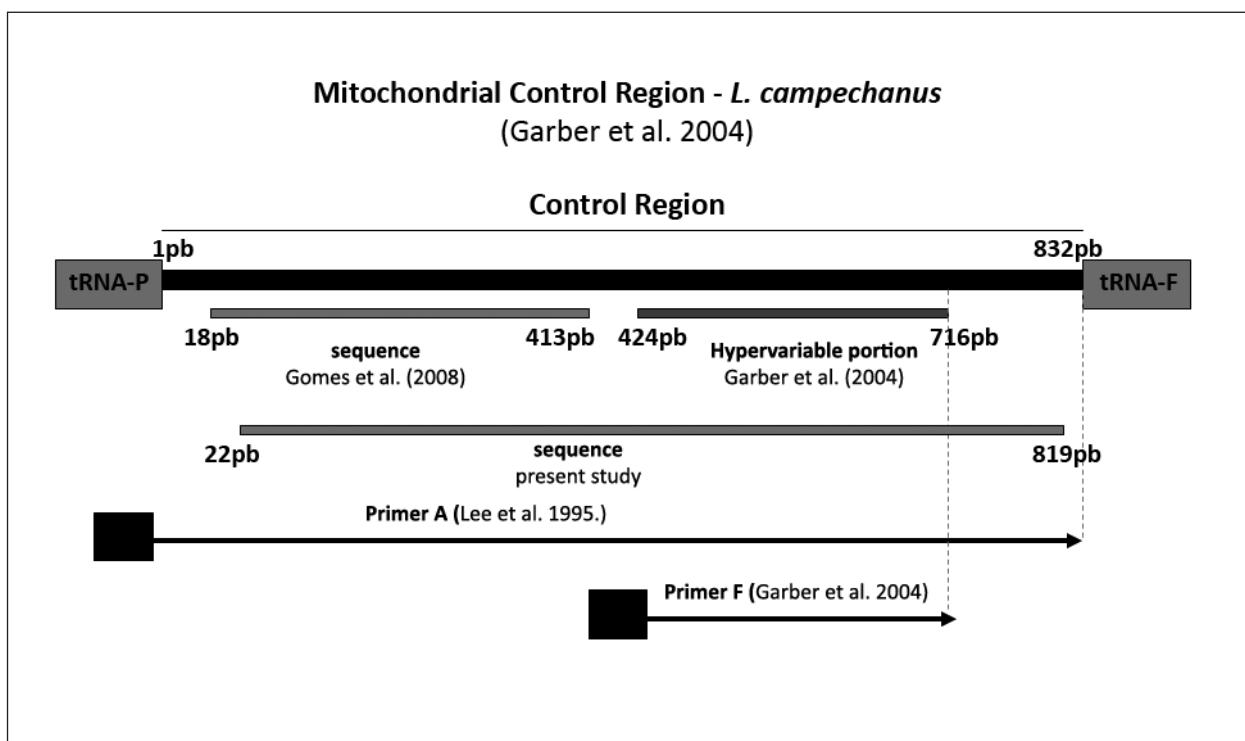


Fig. 2 - Schematic representation of the complete sequence of the mitochondrial control region in *L. campechanus*, showing the fragments sequenced by Gomes et al. (2008) and Garber et al. (2004), and the primers used in the present study.

L. purpureus. The second set of data covered only the hypervariable portion of the control region – a sequence of 299 bp – including both *L. purpureus* and *L. campechanus*.

Saturation of the data was analyzed by DAMBE, version 4.2.13 (Xia and Xie 2001), which plotted mutation rates (transitions and transversions) as a function of the genetic divergence among the sequences.

ANALYSIS OF PHYLOGEOGRAPHIC PROCESSES AND POPULATION GENETICS

The phylogenetic relationships among haplotypes were evaluated through neighbor-joining analyses using MEGA 4.0 (Tamura et al. 2007). The significance of the groupings in all generated trees were estimated using bootstrap analysis, based on 1,000 pseudo-replicates. The distance model used to construct the phylogenetic tree and the nucleotide divergence matrix considered the relative difference between sequences based on pairwise comparisons, the simplest evolutionary model, with no correction for superimposed mutations. The first analysis investigated the phylogenetic relationships of the control region haplotypes of Brazilian *L. purpureus*, while the second approach involved a smaller sequence – the hypervariable portion of the D-loop – but a wider range of samples, through the inclusion of *L. campechanus*. For both analyses specimens of *Lutjanus synagris* collected off the coast of Bahia were used as outgroup.

Additional parameters included the nucleotide composition, and the number of polymorphic and informative sites for parsimony analysis, determined by MEGA 4.0 (Tamura et al. 2007). The number of substitutions (transitions and transversions) was calculated using the Arlequin program (Excoffier et al. 2005). Within-population genetic diversity was evaluated based on the indices of haplotype (*h*) and nucleotide (π) diversity (Nei 1987) provided by the programs DnaSP v 5 (Librado and Rozas 2009) and Arlequin 3.01 (Excoffier et al. 2005). An Analysis of Molecular Variance, AMOVA (Excoffier et al.

1992), based on 20,000 permutations was used to evaluate the proportion of genetic variation within and between *L. purpureus* populations, and between this species and *L. campechanus*. The latter analysis was used to look for the possible existence of distinct genetic stocks in the southwestern (Brazil) and northwestern (USA and Mexico) Atlantic.

Genetic differentiation between pairs of populations was evaluated using the Fixation index, F_{ST} (Weir and Hill 2002), the significance of which was determined using 20,000 permutations, as well as using F/Φ_{ST} of AMOVA. The haplotype network was constructed using the median vector method available in the NETWORK 4.5 program (Bandelt et al. 1999).

DEMOGRAPHIC HISTORY AND NEUTRALITY

The mismatch distribution (number of observed differences between pairs of haplotypes) was used to evaluate the demographic history of the populations of *L. purpureus* and *L. campechanus*. This analysis permits the identification of populations that were stable, expanding, or have passed through bottlenecks in the past (Frankham et al. 2004). When the pairwise differences were plotted on a graph, a unimodal distribution indicates a sudden explosive demographic expansion (Slatkin and Hudson 1991, Rogers and Harpending 1992), whereas stable populations will present an irregular profile due to either the stochastic loss of lineages (Slatkin and Hudson 1991, Aris-Brosou and Excoffier 1996) or secondary contact among populations following a long period of isolation (Frankham et al. 2004). By contrast, populations that have passed through severe bottlenecks with a drastic reduction in genetic variation have either a bimodal or a null distribution, depending on whether the bottleneck only reduced genetic diversity, or eliminated it altogether (Frankham et al. 2004).

The analyses of the mismatch distribution were carried out in Arlequin 3.01 (Excoffier et al. 2005), with the graph being generated in Microsoft

Excel. The mutation parameters before ($\theta_0=2N_0u$) and after ($\theta_1=2N_1u$) expansion were determined using Schneider and Excoffier's (1999) least-square approach, using τ as the estimator of expansion time. The equation $\tau = 2ut$ (Rogers and Harpending 1992) was used to estimate the probable expansion time of the populations, where $u = 2\mu_0K$ (K = number of nucleotides and μ_0 = mutation rate of the genomic region). A mutation rate of 10-13% per million years was used here as the evolutionary rate of the mitochondrial control region in fish (Brown et al. 1993, Zhang et al. 2006), while generation time was set at five years, which corresponds to the age at which female *L. purpureus* reach sexual maturity (R.F.C. Souza, unpublished data, Souza et al. 2003). In addition, the raggedness index (Harpending 1994) and the sum of the squared deviations (SSD) between observed and expected mismatch distributions were calculated. These analyses were also run in Arlequin 3.01 (Excoffier et al. 2005), and were used to validate the estimated expansion model (Schneider and Excoffier 1999). Significance was estimated using a parametric bootstrap analysis.

Fu's (Fs) and Tajima's (D) neutrality tests (Tajima 1989, Fu 1997) were run based on 20,000 simulations, in Arlequin 3.01. Values of both D and Fs were negative when there were an excess of recent mutations, which may indicate recent population expansion, or other evolutionary processes, such as a hitchhiker effect or natural selection (Fu 1997). Positive values may indicate balancing selection, population subdivision or bottlenecks (Tajima 1989).

RESULTS

PHYLOGEOGRAPHY AND POPULATION STRUCTURE IN *L. PURPUREUS*

A total of 810 base pairs (bp) were obtained from the control region of the 239 Brazilian specimens of *L. purpureus* (Fig. 1). Mean nucleotide composition was 30.34% for Thymine, 21.33% for Cytosine,

32.32% for Adenine, and 16.01% for Guanine. Just over a third of the sites (274) were polymorphic, while 527 were conserved. Of the variable sites, 181 were informative for parsimony analysis, while 93 were singletons. A total of 299 substitutions were observed, of which, 260 were transitions, and 39 transversions. The plot of transition/transversion rates vs. genetic distance (not shown) did not indicate the presence of saturation.

No less than 220 haplotypes were detected in the 239 specimens, indicating very high levels of haplotype diversity. The most common haplotype (72) was shared by only five individuals, but was recorded in three populations (CNB I, CNB II and Bahia). No haplotype was recorded in all four populations. In fact, only 13 haplotypes were present in more than one individual, which means that 94.1% of haplotypes were unique, although most were distinguished by a small number of mutations. This situation was also reflected in the predominance (95.5%) of exclusive haplotypes in the four populations. Genetic distances between *L. purpureus* haplotypes varied from 0.1% to 6.1%, while those with the outgroup (*L. synagris*) ranged from 9.8% to 12.3%. The most divergent haplotype (207) was recorded in the Bahia population, and diverged by more than 3.5% in all comparisons. The red snapper populations present considerable genetic variability, considering either the haplotype or nucleotide indices (Table IA). Spatially, the eastern populations (Bahia and Ceará) were relatively more polymorphic, with mean nucleotide diversity of 2.8%, despite their much smaller samples.

In order to test the hypothesis that Brazilian red snappers were divided into distinct genetic stocks, three different approaches were applied for the AMOVA. In the first analysis, the western (CNB I and CNB II) and eastern (Ceará and Bahia) populations were grouped together. Subsequently, the populations CNB and Ceará were separated from that of the eastern coast (Bahia). The third approach involved the allocation of all four populations into

TABLE I

Sample size, number of haplotypes and indices of nucleotide and haplotype diversity for: (A) the mitochondrial control region in Brazilian *L. purpureus*, and (B) the hypervariable portion of the control region in Brazilian *L. purpureus* (present study) and *L. campechanus* sampled by Garber et al. (2004).

Population	N	Number of haplotypes	Haplotype diversity ($\bar{h} \pm \text{sd}$)	Nucleotide diversity ($\chi \pm \text{sd}$)
(A) Mitochondrial control region <i>L. purpureus</i>				
Costa Norte Brasil (CNB I)	119	113	0.999 ± 0.001	0.026 ± 0.013
Costa Norte Brasil (CNB II)	49	49	1.000 ± 0.004	0.025 ± 0.012
Ceará (CE)	43	43	1.000 ± 0.005	0.028 ± 0.014
Bahia (BA)	28	26	0.994 ± 0.011	0.028 ± 0.014
Total	239	220	0.999 ± 0.0006	0.026 ± 0.013
(B) Hypervariable portion <i>L. purpureus</i>				
Costa Norte Brasil (CNB I)	119	95	0.994 ± 0.002	0.031 ± 0.016
Costa Norte Brasil (CNB II)	49	43	0.992 ± 0.006	0.029 ± 0.015
Ceará (CE)	43	42	0.998 ± 0.005	0.033 ± 0.017
Bahia (BA)	28	25	0.992 ± 0.011	0.033 ± 0.017
Total	239	173	0.995 ± 0.001	0.031 ± 0.016
<i>L. campechanus</i>				
Alabama (AL)	06	06	1.000 ± 0.096	0.026 ± 0.016
Cancún (CM)	13	11	0.961 ± 0.049	0.017 ± 0.010
Louisiana (LA)	06	06	1.000 ± 0.096	0.019 ± 0.012
Mississippi (MS and FH-1)	115	80	0.948 ± 0.017	0.020 ± 0.010
Florida (FL)	35	25	0.936 ± 0.034	0.025 ± 0.013
Total	175	109	0.946 ± 0.014	0.020 ± 0.010
Total <i>Lutjanus</i>	414	275	0.985 ± 0.003	0.028 ± 0.000

a single group. In all three approaches, the majority of the variation was found within populations, rather than between populations or groups (Table IIA). Furthermore, the values of F_{st} and F/Φ_{st} considering both the pairwise comparisons and AMOVA, respectively, were low or negative and not significant, indicating intense gene flow among populations. These results point clearly to the existence of a single panmictic population of red snapper in Brazilian waters, extending all along the northern coast and as far south as Bahia. The panmixia of this population was also supported by the phylogenetic tree (due to the large number of haplotypes, the figure is not shown here), in which individuals from different

localities were mixed together in a single clade, completely lacking any phylogeographic structuring. The star-shaped haplotype network (Fig. 3A) further supports the existence of a single genetic stock of Brazilian *L. purpureus*.

The plots of the pairwise differences between haplotypes were almost invariably unimodal, for both individual populations and the sample as a whole, which suggests a process of population expansion (Fig. 4A). The only exception was the population from Bahia, which presented an irregular profile, probably the result of the relatively small size of the sample from this locality. In addition, the values of F_s (Fu 1997) were significantly negative

TABLE II

Analysis of Molecular Variance (AMOVA) for: (A) Brazilian *L. purpureus* (810 bps of the mitochondrial control region); (B) western Atlantic *L. purpureus* and *L. campechanus* (hypervariable portion of the control region). Populations: CBN: Northern Brazilian Coast; CE: Ceará; BA: Bahia; RMS: Mississippi; AL: Alabama; LA: Louisiana; FL: Florida; CM: Cancun, Mexico. Significance level: ns: not significant; * = $P < 0.05$; ** = $P < 0.01$.

Groupings	Variance	% variation	F /Φ
(A) <i>L. purpureus</i> CNB I, II vs. CE, BA			
Between groups	-0.07430 Va	-0.69	F/Φ _{CT} =-0.00688 ^{ns}
Between populations	0.06918 Vb	0.64	F/Φ _{SC} =0.00636 ^{ns}
Within populations	10.80960 Vc	100.05	F/Φ _{ST} =-0.00047 ^{ns}
CNB I, II + Ceará vs. Bahia			
Between groups	0.05781 Va	0.53	F/Φ _{CT} =0.00533 ^{ns}
Between populations	-0.05133 Vb	-0.47	F/Φ _{SC} =-0.00476 ^{ns}
Within populations	10.83626 Vc	99.94	F/Φ _{ST} =0.00060 ^{ns}
All populations			
Between populations	0.02245 Va	0.21	F/Φ _{ST} =0.00207 ^{ns}
Within populations	10.80960 Vb	99.79	
(B) <i>L. purpureus</i> and <i>L. campechanus</i> (RMS, AL, LA) vs. (FL, CM) vs. (CNB I, II) vs. (CE, BA)			
Between groups	0.84621 Va	17.35	F/Φ _{CT} =0.173*
Between populations	0.01416 Vb	0.29	F/Φ _{SC} =0.003 ^{ns}
Within populations	4.01677 Vc	82.36	F/Φ _{ST} =0.176**
Northern Atlantic vs. Southern Atlantic			
Between groups	1.24372 Va	23.67	F/Φ _{CT} =0.236**
Between populations	-0.00583 Vb	-0.11	F/Φ _{SC} =-0.001 ^{ns}
Within populations	4.01677 Vc	76.44	F/Φ _{ST} =0.236**
All populations			
Between populations	0.75075 Va	15.75	F/Φ _{ST} =0.157**
Within populations	4.01677 Vb	84.25	

for all populations, which indicates the non-neutral nature of the marker used here, and supports the hypothesis of demographic expansion. The values of D (Tajima 1989) were all negative, although they were only significant for the two western (CNB) populations and the sample as a whole (Table IIIA). These results also support the idea that the Brazilian red snapper population is undergoing expansion, as well as indicating the occurrence of recent mutations and haplotypes with very low frequencies. These

conclusions are also supported by the SSD and raggedness index (Harpending 1994). Given the mutation rate selected (10-13% per million years), the values of μ vary from 5.0×10^{-8} to 6.5×10^{-8} , and considering the 810 bps sequenced here and a generation of five years, values of u ranged from $20,250 \times 10^{-8}$ to $26,325 \times 10^{-8}$. The estimated value of τ was 23,162, which indicates that the expansion of the Brazilian population of red snappers took place approximately at 220,000 to 286,000 years ago.

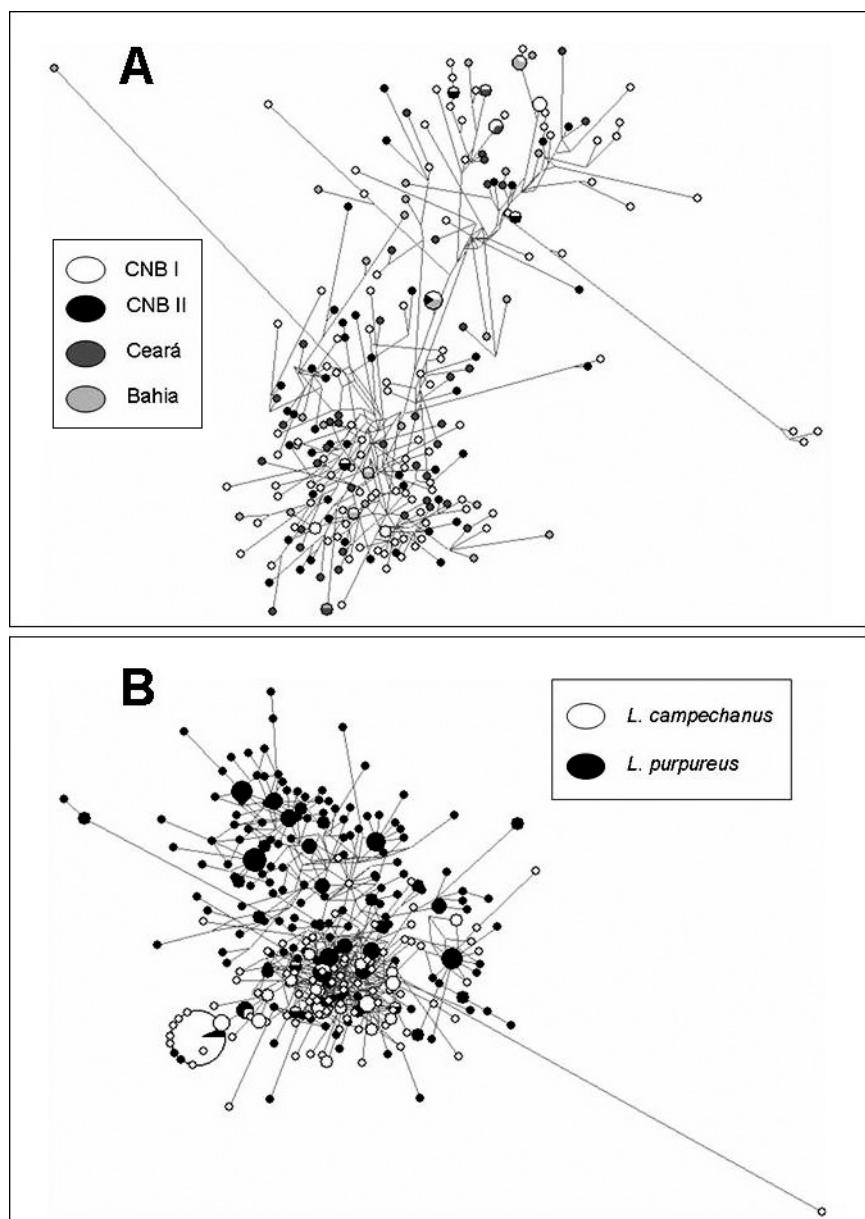


Fig. 3 - Haplotype networks based on (A) the mitochondrial control region (810 bps) of Brazilian *L. purpureus*, and (B) the hypervariable portion of the control region in *L. purpureus* and *L. campechanus*.

L. PURPUREUS AND *L. CAMPECHANUS*: ONE OR TWO SPECIES?

The 299 bp sequence corresponding to the hypervariable portion of the control region was analyzed in 414 individuals representing *L. purpureus* ($n=239$) and *L. campechanus* ($n=175$) from a range of localities (Fig. 1). The *L. campechanus* sequences (Garber et al. 2004) were obtained from GenBank (access codes: AF356881-

7004; AF356750-776; AY153500-23). The hyper-variable region described by Garber et al. (2004) was located in the second half of the control region, roughly between nucleotides 420 and 720 (Fig. 2).

The mean overall nucleotide composition (considering all 414 specimens) was 32.2% for Thymine, 20.8% for Cytosine, 26.3% for Adenine, and 20.7% for Guanine. As for the control region

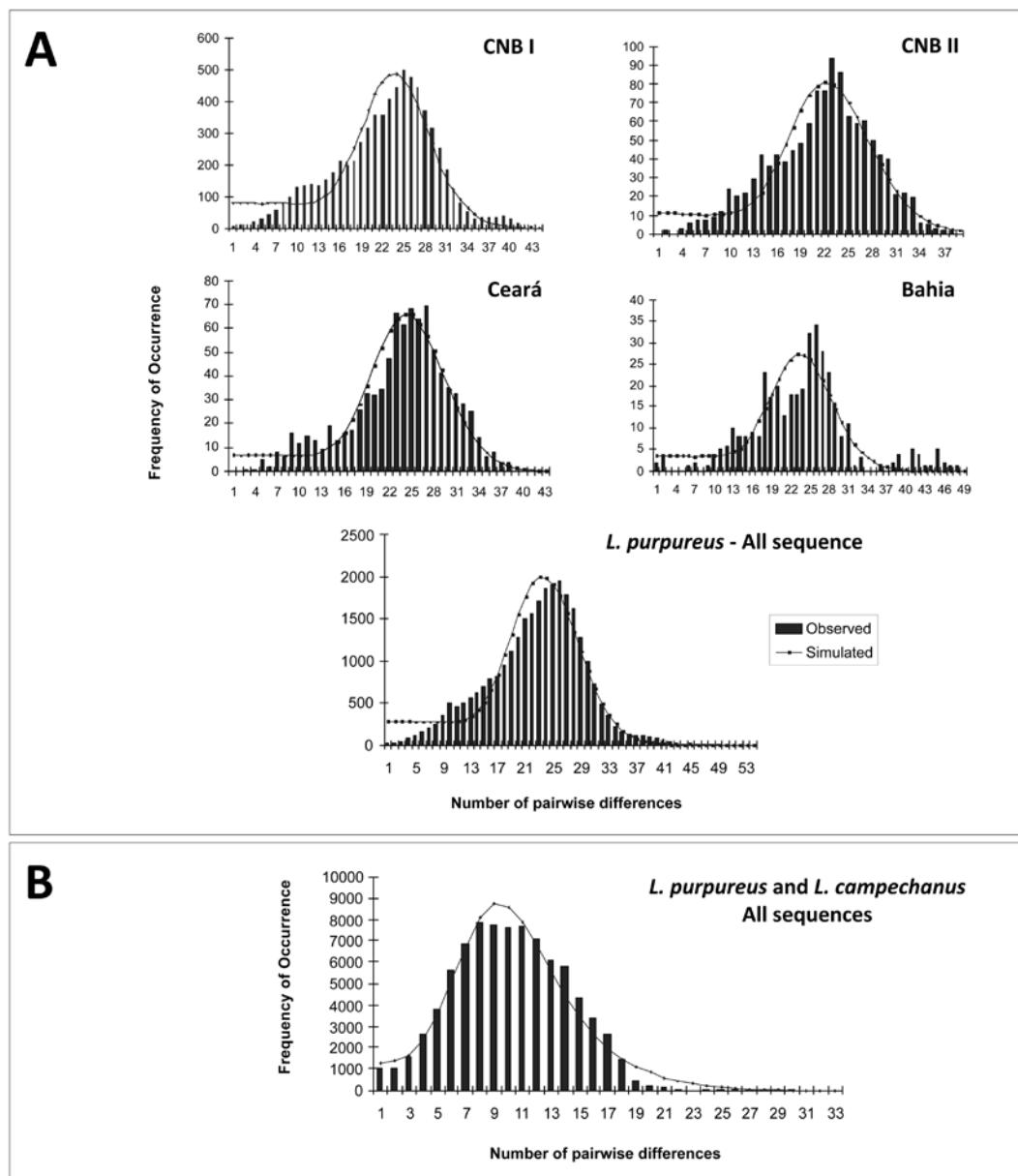


Fig. 4 - Distribution of pairwise differences between haplotypes based on sequences of the mitochondrial control region in: (A) *L. purpureus* from four Brazilian localities (CNB I, CNB II, Ceará, and Bahia) and all sequences, and (B) *L. purpureus* and *L. campechanus* ($n = 414$) from the western Atlantic.

as a whole, just over a third (101) of sites were polymorphic, while 195 were conserved. Sixty-three of the sites were informative for parsimony analysis, while 38 were singletons. There were 122 substitutions, 94 of which were transitions, and 28 transversions.

A total of 275 haplotypes were observed in the 414 specimens. When the sites containing deletions

were omitted from the analysis, this total dropped to 240, although the overall pattern of frequency and distribution was maintained. The most common haplotype (14) was shared by 43 individuals representing all populations except CNB II and Ceará, while it was most abundant in Mississippi and Florida. Others, such as haplotypes 1, 4, 6, and 17, were found in three to seven individuals,

TABLE III

Results of the analysis of the pairwise differences (mismatch) between the control region haplotypes of:

(A) Brazilian *L. purpureus*, and (B) western Atlantic *L. campechanus* and *L. purpureus*.

Significance level: ns: not significant; * = $P < 0.05$; ** = $P < 0.01$.

<i>Population</i>	<i>N</i>	τ	θ_0	θ_1	<i>Variance</i>	<i>SSD</i>	<i>P</i>	<i>Raggedness</i>	<i>P</i>	<i>Fs</i>	<i>D</i>
(A) <i>L. purpureus</i>											
CNB I	119	23,971	0.063 (0,000-5,998)	87,544 (41,548-99999,000)	50,557	0.0013	0.6950	0.0009	0.9980	-23.894**	-1.544*
CNB II	49	20,625	2,215 (0,000-12,052)	106,948 (52,573-99999,000)	41,357	0.0017	0.7360	0.0018	0.9850	-24.157**	-1.458*
Ceará	43	24,785	0.005 (0,000-6,291)	129,580 (60,233-99999,000)	45,012	0.0013	0.9090	0.0022	0.9730	-24.067**	-1.332 ns
Bahia	28	23,734	0.077 (0,000-6,253)	106,147 (48,171-99999,000)	61,046	0.0058	0.4280	0.0066	0.6790	-7.360*	-1.396 ns
Total	239	23,162	0.775 (0,000-5,955)	96,714 (54,292-99999,000)	49,553	0.0010	0.6410	0.0008	0.9930	-23.630**	-1.704**
(B) <i>L. purpureus</i> and <i>L. Campechanus</i>											
<i>L. purpureus</i>	239	9,088	1,092 (0,000-5,486)	69,453 (30,547-99999,000)	13,284	0.0005	0.7470	0.0029	0.9020	-24.189**	-1.086 ns
<i>L. campechanus</i>	175	6,742	0.004 (0,000-3,143)	29,966 (12,284-99999,000)	13,195	0.0015	0.6830	0.0081	0.7230	-24.905**	-1.434*
Total	414	7,414	2,858 (0,000-13,897)	65,566 (23,496-99999,000)	16,963	0.0009	0.5940	0.0022	0.9290	-23.997**	-1.275 ns

but overall, only seven haplotypes were shared by the two species, while the others were exclusive to either *L. purpureus* (approximately 60% of the total) or *L. campechanus* (37%). However, most of these haplotypes were distinguished by a small number of mutations.

Genetic divergence between *L. purpureus* and *L. campechanus* haplotypes varied from 0.4% to 9.3% (average of 2.9%), and the most divergent haplotype (272) was from Florida. Most haplotypes varied by less than 3%, however, even when comparing species such as the value of 0.7% recorded between individuals of the Mississippi and western Brazilian populations. These results and their implications are very similar to those found

above for the control region (810 bps) in Brazilian *L. purpureus*. Genetic variation were relatively high, whether considering either haplotype or nucleotide diversity. This variation was greatest in the Brazilian *L. purpureus*, in particular the eastern populations, in which nucleotide diversity values were above 3%. Overall, however, the pattern of genetic diversity was similar in the two species (Table IB).

Once again, three different approaches were used for the AMOVA (Table IIB). The first was based on four main geographic groups – the western (CNB I and II) and eastern (Ceará and Bahia) Brazilian groups, a Gulf group (Alabama, Louisiana, and Mississippi), and a Caribbean group (Florida and Cancún). In the second analysis, the

specimens were grouped by species, representing the northwestern (*L. campechanus*) and southwestern (*L. purpureus*) Atlantic. The final approach was to include all the specimens in a single group.

As for the previous analysis of the complete control region in *L. purpureus*, the majority of the variation recorded in all three approaches was found within, rather than between groups. However, a moderate, but significant process of differentiation was observed between the two species ($F/\Phi_{CT} = 0.236$, $P < 0.01$; see Table II), with significant values of F/Φ_{ST} ($F/\Phi_{ST} = 0.176$; 0.236 , $P < 0.01$; see Table II). The pairwise F_{st} comparisons reconfirmed panmixia in the Brazilian snappers, with low or negative (non significant) values between all populations. A similar situation was recorded for *L. campechanus* (Table IV). However, when comparisons were made between species, the F_{st} values were significant in all cases except Louisiana vs. Bahia, possibly because of the reduced sample sizes for these populations. These results suggest a moderate degree of differentiation between the two species, despite their considerable similarities and sharing of haplotypes.

As before, the phylogenetic tree (not presented here because of the large number of haplotypes) was characterized by a complete mixing of individuals

from the two species, resulting in the formation of a single clade with countless polytomies and reduced statistical support. This confirmed the absence of any systematic phylogeographic structuring between *L. purpureus* and *L. campechanus*, which was further reinforced by the star-shaped haplotype network (Fig. 3B).

The plot of the pairwise differences between haplotypes for the whole sample ($n = 414$) revealed a unimodal distribution characteristic of historical population expansion (Fig. 4B). This was confirmed by the values of τ , θ_0 and θ_1 , SSD and the raggedness index (Table IIIB). In addition, the F_s values were negative and significant for all the populations, rejecting the neutrality of the marker used here, and emphasizing the process of population expansion. The D values were negative for all the populations, but were only significant for *L. campechanus* (Table IIIB).

Estimates of population expansion were based on the same criteria used above for *L. purpureus*. However, as the two species have different ages of maturation, the calculations were conducted using both values, i.e. 2 years for *L. campechanus* (Moran 1988) and 5 years for *L. purpureus* (R.F.C. Souza, unpublished data, Souza et al. 2003). Using the same mutation rate as before, values of μ vary

TABLE IV
Matrix of F_{st} values for pairwise comparisons between populations of *L. purpureus* and *L. campechanus* from the western Atlantic. Significance level: ns: not significant; * = $P < 0.05$; ** = $P < 0.01$.

	<i>L. purpureus</i>				<i>L. campechanus</i>			
	CNBI	CNBII	Ceará	Bahia	Mississippi	Alabama	Louisiana	Florida
CNBII	0.010 ^{ns}							
Ceará	-0.004 ^{ns}	-0.007 ^{ns}						
Bahia	-0.001 ^{ns}	0.013 ^{ns}	0.001 ^{ns}					
Mississippi	0.238**	0.324**	0.295**	0.198**				
Alabama	0.175**	0.248**	0.203**	0.117*	0.039 ^{ns}			
Louisiana	0.118**	0.193**	0.148**	0.061 ^{ns}	-0.001 ^{ns}	-0.027 ^{ns}		
Florida	0.192**	0.266**	0.232**	0.144**	-0.003 ^{ns}	0.007 ^{ns}	-0.005 ^{ns}	
Cancun	0.206**	0.290**	0.251**	0.158**	-0.023 ^{ns}	0.000 ^{ns}	-0.003 ^{ns}	-0.027 ^{ns}

from 5.0×10^{-8} to 6.5×10^{-8} . Considering a generation time of 5 years and the 299 bps sequenced here, μ ranged from $7,475.0 \times 10^{-8}$ to $9,717.5 \times 10^{-8}$. The value of τ was estimated to be 7,414, indicating that population expansion took place between approximately 191,000 and 248,000 years ago. The same result was obtained when a generation time of 2 years was considered.

DISCUSSION

BRAZILIAN *L. PURPUREUS*

Phylogeography and Population Structure

The species *L. purpureus* represents an important fishery resource throughout its distribution in the western Atlantic, although little is known of the genetic structure of its populations, despite the value of such knowledge for the management of stocks. The results of the present study reconfirm the findings of Gomes et al. (2008), and indicate that the Brazilian populations of red snapper form a single genetic stock, characterized by intense internal gene flow, contradicting the previous study of Salles et al. (2006), which also used mitochondrial markers, and indicated the existence of two geographically distinct stocks. These authors considered the western stock to be less productive, given the presence of smaller reproductively mature individuals. Unfortunately, this apparent, however unexpected discrepancy between the topologies retrieved by CytB region and control can not be tested because the sequences CytB cited by Salles et al. (2006) are not available in Genbank. However, as the authors used only 12 specimens and a fragment with only 307 base pairs, we postulate that this discrepancy is due to small sample size and the short DNA fragment.

In addition, our molecular findings contrasts with the morphometric and meristic analyses performed by Sousa-Júnior et al. (2002) and Salles et al. (2006). This discrepancy cannot be easily explained, except by the

fact that the two data sets (molecular and morphology) experience different selective forces. However, only a combined analysis, which was not possible in this approach, may be quite clear on this issue.

Regarding the migratory cycle proposed by Ivo and Hanson (1982), our interpretation is that, irrespective of the cohorts number, the juveniles mingle in the breeding area, prior to being recruited to the feeding areas on the continental shelf.

The pattern of marked genetic homogeneity recorded here for *L. purpureus* appears to be typical of western Atlantic lutjanids, such as *L. campechanus* and *Rhomboplites aurorubens* in the Gulf of Mexico and Florida (Bagley et al. 1999, Garber et al. 2004) and *Ocyurus chrysurus* in Brazil (Vasconcellos et al. 2008). These species are behaviorally similar, demersal inhabitants of relatively deepwater habitats with rocky bottoms and/or coastal regions close to coral reefs (Allen 1985). They also share pelagic development stages, during which their eggs and larvae are transported by marine currents, which generally limit the potential for speciation. This type of reproductive process results in an extensive intermingling of individuals, which presumably influences the genetic connectivity among populations.

However, other lutjanid species present very different genetic characteristics, with marked divergence among populations. These species include *Pristipomoides multidens* (Ovenden et al. 2004), *Lutjanus erythropterus* (Zhang et al. 2006), and *L. synagris* (Karlsson et al. 2009). Other perciforms, such as the king weakfish, *Macrodon ancylodon*, also present considerable genetic differentiation at the population level (Santos et al. 2003, 2006). These authors recorded the presence of two distinct genetic stocks of *M. ancylodon* along the Atlantic coast of South America, which they interpreted as the result of a process of allopatric differentiation. One of the determining factors in this case may be differences in water temperature resulting from the action of oceanic

currents (Santos et al. 2006). Other authors, such as Rocha et al. (2004) have related environmental variables to distribution patterns and differentiation in marine fish species, finding that populations may be connected over thousands of kilometers, where similar habitats exist, as in the case of *L. purpureus* on the Brazilian coast. It is nevertheless important to bear in mind that, in addition to ecological variables, historical factors and associated evolutionary processes may also contribute to the present-day configuration of geographic ranges in the western Atlantic (Rocha 2003).

Genetic variability and divergence

The Brazilian populations of red snapper present high levels of genetic variation, considering either haplotype or nucleotide diversity. Unique haplotypes or haplotypes exclusive to a given population predominate. A similar pattern has been observed in other commercially-important lutjanid species, such as *L. campechanus* (Garber et al. 2004), *L. erythropterus* (Zhang et al. 2006), and *O. chrysurus* (Vasconcellos et al. 2008). The relative abundance of unique haplotypes – a pattern common to all these species – may mean that many alleles are lost through harvesting, which will eventually affect the genetic diversity of stocks.

It is interesting to note that the highest levels of nucleotide diversity were recorded in the eastern populations (Ceará and Bahia). This may be related to the decline in fishery activities in this region (Ivo and Sousa 1988, Paiva 1997), which would have partially released local populations from this predatory pressure. On the other hand, as the Brazilian population appears to be panmictic, this spatial variation may be related to ecological factors, such as the abundance of coral reefs and oceanic banks off the coast of the Brazilian Northeast. *L. purpureus*, a nektonic demersal species (Szpilman 2000), appears to prefer relatively deep water (30–60 m) (Allen 1985) with a rocky or coralline bottom,

with juveniles generally inhabiting shallower areas. However, commercial fishing of the species in Brazil is relatively recent – around 50 years (Fonteles-Filho 1972, Paiva 1997) – which may be a too short period for significant spatial and temporal variations in the diversity of the species to occur.

Divergence between haplotypes varied from 0.1% to 6.5%, although in many cases, the value was below 2%, which indicates haplotypes distinguished by a small number of mutations. In other species of the genus, such as *L. campechanus*, divergence between haplotypes is typically much higher, reaching 9% (Garber et al. 2004).

Demographic History

All the populations studied here, with the exception of that from Bahia, presented a smooth unimodal distribution in the plot of pairwise differences, which indicates exponential growth, or population expansion (Frankham et al. 2004). The estimated expansion time of Brazilian *L. purpureus* coincides with the late Pleistocene, as confirmed by Gomes et al. (2008). Pruett et al. (2005) also proposed a Pleistocene expansion for the population of *L. campechanus*.

The star-shaped haplotype network, and other parameters (SSD, raggedness index) also indicate a process of expansion in the Brazilian red snapper population, which is further confirmed by the θ intervals, reflecting considerable differences in the size of the population before and after the expansion process. It seems likely that the irregular distribution observed in the plot for Bahia is related primarily to the small size of the sample from this area, given that other analyses indicated expansion in this population, although it may also reflect the relatively ample variance observed in the mismatch distribution.

Garber et al. (2004) recorded a similar pattern in populations of *L. campechanus*, once again with a single exception (Florida), possibly for reasons similar to those of the *L. purpureus* population

from Bahia. Similar results have also been obtained for *L. erythropterus* (Zhang et al. 2006) and *Thallasoma hardwicki* (Chen et al. 2004).

Significant negative values of *F_s* and *D* were recorded for the Brazilian red snapper populations analyzed here, except for *D* in the Ceará and Bahia populations. These results confirm that the marker used here is non-neutral, and also suggest haplotypes of low frequency distinguished by few mutations, as well as an accumulation of new mutations (Tajima 1989, Fu 1997). The significant negative values of *F_s* also indicate population expansion in the recent past, as observed in other marine fish, such as *O. chrysurus* (Vasconcellos et al. 2008), *L. erythropterus* (Zhang et al. 2006), *C. acoupa* (Rodrigues et al. 2008) and *T. hardwicki* (Chen et al. 2004).

RED SNAPPERS OF THE WESTERN ATLANTIC: *L. PURPUREUS* AND *L. CAMPECHANUS*

One or Two Species?

Mayr's (1963) biological species concept is based on the idea of the reproductive isolation of populations, and is the most widely-accepted definition of a species, in particular in areas such as population genetics, evolutionary biology, and conservation biology (Frankham et al. 2004). The situation observed in the red snappers *L. purpureus* and *L. campechanus* is characteristic of that of a single species, considering not only the homogeneity of morphological traits (Allen 1985, Moran 1988, Cervigón 1993, Cervigón et al. 1993, De-la-Rosa 2001, Wilson and Nieland 2001, R.F.C. Souza, unpublished data, Souza et al. 2003), but also the marked similarity in genetic features (Gomes et al. 2008, present study).

However, there is a consensus in the literature that the geographic ranges of these two snappers are not sympatric. Many authors, such as Cervigón (1993) and Allen (1985) believe that these species

are parapatric in the western Atlantic, with *L. campechanus* being restricted to the Gulf of Mexico and Atlantic coast of the United States, and absent from the Caribbean (Rivas 1966, Allen 1985), whereas *L. purpureus* is found south of Cuba, southwards throughout the Caribbean to Bahia in northeastern Brazil (Allen 1985, Cervigón 1993). Some authors (Rivas 1966, Carpenter and Nelson 1971) nevertheless suggest that *L. purpureus* also occurs in some parts of the coast of the United States, in which case, contact between populations, and gene flow, would be possible. Either way, the restricted ranges of the two species contrasts considerably with those of other western Atlantic snappers, such *L. vivanus*, *L. synagris* and *L. buccanella*, which present a continuous distribution along the eastern seaboard of the New World (Allen 1985, Cervigón 1993).

The analysis presented here demonstrated that *L. campechanus* and *L. purpureus* share mitochondrial haplotypes, indicating the occurrence of gene flow between their populations. The AMOVA also found that the majority of the variance was within rather than among populations or geographic groups, even though the *F_{ST}* values for pairwise comparisons between species were significant. The magnitude of the recorded values ($F/\Phi_{ST} = 0.236, P < 0.01$) indicates only moderate genetic differentiation between the two snapper species, and may simply reflect the elevated polymorphism of the control region in these fish. Similar values were recorded in intra-population comparisons in lutjanids, including species from the western Atlantic (Ovenden et al. 2004, Vasconcellos et al. 2008).

Other aspects of the results also support the hypothesis that only a single species of red snapper exists in the western Atlantic, such as the star-shaped haplotype network and the absence of phylogeographic structuring in the phylogenetic tree, which clearly indicates the existence of a single monophyletic group of red snappers, a

strong evidence for the existence of a single species (Cracraft 1983). A similar conclusion was obtained in the preliminary analysis of Gomes et al. (2008).

The sum of the evidences appears to indicate that *L. campechanus* and *L. purpureus* actually represent a single species of red snapper with moderately restricted gene flow. The two species clearly share a common evolutionary history, but given the nature of the marker used here (haploid, with strictly maternal inheritance) (Brown 2008), it may be necessary to consider alternatives explanations for the observed scenario.

One possibility is that the gene flow detected between populations reflects the incomplete separation of lineages, with the retention of an ancestral polymorphism. This would explain the absence of reciprocal monophyly between the two species, and may indicate that the cladogenetic event which rose the two species was relatively recent, given the fact they still share the same mitochondrial lineage. A second alternative is the possibility of hybridization or introgression, resulting from the generation of a fertile hybrid, which subsequently breeds with members of one or both of the original species, resulting in gene flow between them. Such events were well documented (Freyhof et al. 2005, Sanz et al. 2006, Castillo et al. 2008). This process would nevertheless depend on the presence of a contact zone between the ranges of the two species, which has yet to be confirmed in the present case. Hybridization was recorded in a number of lutjanid species, such as *L. synagris* and *O. chrysurus* (Domeier and Clarke 1992, Loftus 1992). Recent analyses (Rocha and Molina 2008, Nirchio et al. 2008, 2009) have confirmed that the chromosomal constitution of the lutjanids, including species of the genera *Lutjanus*, *Ocyurus*, and *Rhomboplites*, is highly conserved, which would permit introgressive hybridization or hybridization. At the moment, however, there is no evidence of such hybridization in the red snappers. Confirmation of any of these alternatives would

require a more reliable evaluation of gene flow based on the analysis of additional mitochondrial loci and nuclear genes.

The absence of genetic sub-structuring among populations of marine fish was recorded in a wide range of western Atlantic fish species, including other lutjanids (Garber et al. 2004, Santa Brígida et al. 2007). One of the possible explanations for this phenomenon is the considerable dispersal potential of the pelagic larval forms, which can be transported over long distances by ocean currents. The dimensions and relative continuity of the marine environment in this region may be an additional factor.

Patterns of Genetic Variation and Demographic

The populations of red snappers analyzed in the present study presented high levels of genetic polymorphism. The overall pattern of variation was similar in the two species, although diversity was greater in the Brazilian *L. purpureus*. The species could not be differentiated based on indices of genetic variability. In addition, many parameters of interspecific genetic distance were lower than those recorded within populations of both species (Garber et al. 2004, Gomes et al. 2008, present study) and also other lutjanids (Ovenden et al. 2004).

Despite not analyzing the hypervariable portion of the control region, Gomes et al. (2008) recorded a similar pattern in these species. Grant and Bowen (1998) have argued that elevated haplotype and nucleotide diversity may be evidence of a stable population with a long evolutionary history.

As both red snappers are gonochoric and oviparous, and lack sexual dimorphism (De-la-Rosa 2001, R.F.C. Souza, unpublished data, Souza et al. 2003), we conclude that cohorts of both species have distinct breeding areas, with the intense intermingling of individuals occurring after fertilization, during the initial stages of development, a process facilitated by ocean currents. Considering a single species of western Atlantic red snapper, as suggested by the molecular

data, it would seem likely that an extensive area of intermingling exists, which would be responsible for the elevated levels of genetic polymorphism and the similarities in the patterns of variation observed in the populations analyzed here.

When the two red snappers were combined for analysis, the plot of the pairwise differences between haplotypes revealed a unimodal distribution, characteristic of a population that has undergone expansion in the past (Slatkin and Hudson 1991, Rogers and Harpending 1992), as observed by Gomes et al. (2008). This conclusion is also supported by other parameters, such as the low and non-significant SSD and raggedness index, and the significantly negative value of F_s . The same pattern was found in both species individually (Garber et al. 2004, present study), and in other lutjanids, such as *L. erythropterus* (Zhang et al. 2006). The estimated expansion time of *L. campechanus* coincides with the late Pleistocene, as reported by Pruett et al. (2005).

IMPLICATIONS FOR THE CONSERVATION OF THE SPECIES

Reliable data on geographic range and genetic variability are crucial to the development of successful conservation and management strategies for any species, but especially those which suffer intense anthropogenic impacts, as in the case of the red snapper. Information derived from the analysis of DNA fragments has been increasingly employed in conservation programs. In the specific case of the fish ichthyofauna, a large number of studies have confirmed the effectiveness of the mitochondrial control region for the detection of gene flow between populations or species, and the understanding of genetic variation, phylogeographic patterns, and demographic history (Garber et al. 2004, Sanz et al. 2006, Zhang et al. 2006, Gomes et al. 2008, Rodrigues et al. 2008, Vasconcellos et al. 2008).

In the present study, the analysis of the control region of populations of Brazilian *L. purpureus* revealed the existence of a single genetic stock,

which is an extremely important finding for the management of fishery stocks. In addition, high levels of genetic variation and a predominance of unique and exclusive haplotypes were found. This pattern of haplotype distribution may reflect an imbalance of the species genetic diversity, which could require the implementation of effective management strategies that integrate the molecular data with those on ecological variables and reproductive parameters.

Together with data from previous analyses (Gomes et al. 2008), the results of the present study indicate emphatically that *L. purpureus* and *L. campechanus* represent different populations of the same species, with an ample geographic distribution stretching from the southeastern United States to northeastern Brazil. However, as the genetic marker used here represents a single locus with maternal inheritance, we cannot rule out other possible explanations for the observed gene flow between species, such as introgression, hybridization or the retention of an ancestral polymorphism.

While the results of the molecular analysis presented here indicate the existence of a single, genetically diverse species, a more reliable evaluation would include nuclear genes. Irrespective of their taxonomic status, if each species represents a panmictic population within its own geographic range, as suggested here and in previous studies (Gold et al. 2001, Garber et al. 2004, Gomes et al. 2008), their management as separate stocks may be the most effective approach over the long term. Such management is already in place for *L. campechanus*, but in the case of *L. purpureus*, there is an urgent need for the implementation of measures that will guarantee the maintenance of the genetic diversity of the species, and impede the loss of alleles.

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RESUMO

Para o presente estudo utilizou-se a região controle mitocondrial para investigar os padrões filogeográficos e a estrutura populacional de *Lutjanus purpureus* e para avaliar a similaridade genética entre *L. purpureus* e *L. campechanus*. Para a análise inicial foram obtidas sequências de 810 pares de bases (pb) da região controle para 239 espécimes de *L. purpureus* de quatro localidades da costa brasileira. Os resultados mostraram a presença de uma população panmítica caracterizada por altos valores de diversidade genética. Utilizou-se um segmento de 299 pb da porção hipervariável para comparar *L. purpureus* e *L. campechanus*. Dos 414 indivíduos analisados foram identificados 275 haplótipos. A árvore filogenética e a rede de haplótipos não mostraram subestruturação filogeográfica entre as duas espécies, com intensa mistura de indivíduos. Considerando a grande similaridade morfológica, os dados moleculares apresentados, aqui indicam que apenas uma única espécie de pargo vermelho existe ao longo do atlântico ocidental.

Palavras-chave: região controle, *Lutjanus purpureus*.

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