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Short Communication

Can *Lutjanus purpureus* (South red snapper) be "legally" considered a red snapper (*Lutjanus campechanus*)?

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Abstract

Red snappers (*Lutjanus purpureus* in Brazil and *Lutjanus campechanus* in USA and Gulf of Mexico) are both under clear effect of overfishing. Because of their high morphological similarity it has already been suggested that they could possibly be considered as a single species. To investigate the degree of similarity and the genetic structure of red snapper populations we constructed a common dataset of partial D-loop mtDNA sequences of *L. purpureus* from Brazil (Amapá, Pará and Maranhão) and *L. campechanus* from the Atlantic coast of the USA (Florida, Louisiana and Mississippi). Phylogenetic and population genetic analyses surprisingly depicted high similarity between *L. campechanus* and *L. purpureus,* compatible with the hypothesis of a single species of red snapper for the Western Atlantic Ocean. These preliminary but very curious findings open an important discussion regarding the legislation involved on the capture of this overexploited fish resources as well as regarding their taxonomy.

Key words: red snapper, *Lutjanus purpureus*, *Lutjanus campechanus*, population genetic structure, mitochondrial D-loop.

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Lutjanus campechanus (northern red snapper) and *Lutjanus purpureus* (southern red snapper) are very important fish resources which are heavily fished along their distribution areas in the Western Atlantic Ocean (Rivas, 1966; Cervigón, 1993). *L. campechanus* populations are spread on the East Coast of the USA, from North Carolina to Florida including the entire Gulf of Mexico (Rivas, 1966). According to Rivas (1966) and Carpenter and Nelson (1971), *L. purpureus* is found in the Caribbean Sea, in some areas of the USA coast, and also can be found on the northern and northeastern coast of Brazil. Cervigón (1993) reports a distribution of *L. purpureus* from south of the Antilles to Northeastern Brazil.

The Brazilian Ministry for Environment has registered for the year of 1975 an amount of capture of *L. purpureus* in Brazil close to nine thousand tons. Despite improvement in fleet and fishery techniques, the production decreased seven years later to only six thousand tons, which was an indication of overfishing of southern red snappers. A more dramatic scenario has been described in USA for the northern red snapper, *L. campechanus* during the last decades. In 1996 the Gulf of Mexico Fishery Management Council and the US Department of Commerce declared that *L. campechanus* was grossly overfished and called for strict management measures to restore stocks to sustainable levels (Garber *et al.*; 2004; Marko *et al.*; 2004)

There is a remarkable similarity in morphology between *L. campechanus* and *L. purpureus,* starting by their same red color pattern, and coupled with completely identical (hard and soft dorsal and anal fin rays) or overlapping (hard and soft pectoral fin rays, scale on lateral line, and gill rakers) meristic characters. Based on these characters, Cervigón (1993) raised the hypothesis of the existence of a single species of red snapper for the western Atlantic Ocean.

A recent phylogenetic analysis based on mitochondrial DNA sequences (Marko *et al.* 2004) surprisingly revealed that 77% of the fish sold in the marketplace in USA labeled as red snapper (*Lutjanus campechanus*) belonged indeed to other species of the Lutjanidae family, such as *Lutjanus erythropterus*, *L. synagris* and *Rhomboplites aurorubens*. According to US FDA (Food and Drug Administration) (see Marko *et al.*, 2004) only *L. campechanus* can be legally labeled in USA as red snapper, regardless of the additional species called as red snappers in different countries, which is the case for *L. purpureus* in Brazil. However, if the hypothesis of Cervigón (1993) that *L.*

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campechanus and *L. purpureus* are not different species became confirmed, all issues related to stock management of red snappers must be reconsidered.

To make a contribution to this interesting debate, we carried out the first molecular comparative analysis between these two red snappers. For this purpose we collected DNA sequences of about 400 bp for the mitochondrial control region (D-loop) from 93 specimens of *L. purpureus* captured in three distinct localities of northern coastal waters of Brazil (Amapá- 3° 20' N, 50° 41' W; Pará- 0° 08' N, 47° 32' W and Maranhão- 2° 13' S, 42° 39' W). These sequences were pooled together in the same alignment with D-loop sequences for 27 *L. campechanus* deposited in GenBank (Garber, 2001) for Florida (AF356750-AF356757), Louisiana (AF356758-AF356763) and Mississippi (AF356764-AF356776).

To extract DNA, muscle tissue was for 1 h by ribonuclease at 37 °C, followed by 2-4 h incubation at 55 °C with proteinase K. Total DNA was then purified by standard phenol/chloroform extraction followed by precipitation using isopropanol (Sambrook *et al.*, 1989). For each DNA sample the entire D-loop was amplified using the primers L1-5'-CCTAACTCCCAAAGCTAGGTATTC-3' and H2-5'-CCGGCAGCTCTTAGCTTTAACTA-3' designed for this work, and a fragment of approximately 400 base pairs was sequenced. PCR reactions were performed in 25 µL of reaction mixture containing 4 µL of 1.25 mM dNTP, 2.5 µL of buffer (10X conc.), 1 µL of 25 mM MgCl2, 0.5 µL of each primer (200 ng/µL), 1 µL of total DNA (200 ng/µL), 0.5 µL of 5 U/µL Taq DNA polymerase and 15 µL of pure water to complete the final volume of 25 µL. Amplifications were performed in a thermocycler with a cycling profile of 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 57 °C for 1 min, 72 °C for 2 min, ending with an extension step at 72 °C for 7 min. Amplification products were purified using ExoSap IT (Amersham-Pharmacia Biotech. Inc., Piscataway, NJ, USA) and submitted to a cycle-sequencing reaction using the fluorescence-labeled dideoxy terminators supplied in the ABI PrismTM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were performed in a 10 µL reaction mixture containing 1 µL of DNA, 0.5 (1 mM) of primer, 1 µL of Big Dye mix, 2 µL of buffer (200 mM Tris/5 mM MgCl2) and 5.5 µL of pure water. We used a cycling profile of 25 cycles of 96 °C for 30 s, 50 °C for 15 s and 60 °C for 3 min. The products were separated by electrophoresis (3 h at 3.000 V) and the sequences collected using the ABI Prism 377 automated sequencer. Sequences were aligned using the Clustal X version 1.8 program (Thompson *et al.*, 1997) with default parameters. Minor modifications were made using the BIOEDIT sequence editor (Hall, 1999). Sequences of *Lutjanus synagris* (Lane Snapper) from Pará State (Brazil) were used as outgroup. All newly generated D-loop sequences were deposited in GenBank (accession numbers EF656482-EF656576).

The best fit model selected by hierarchical Likelihood Ratio Test in Modeltest Version 3.6 (Posada and Crandal, 1998) was HKY+I+G with a proportion of invariant loci equal to 0.3934 and rates modeled by a gamma distribution with parameter alpha equal to 0.701. A Neighbor Joining tree was built in PAUP* (Swofford, 2002) using the parameters chosen by Modeltest, and 1000 bootstrap pseudoreplicates were generated in order to establish node reliability. The analysis of molecular variance (AMOVA) was used to partition genetic variance within and between populations of northern and southern red snapper using Arlequin 3.0 (Excoffier *et al.*, 2005). The exact test of sample differentiation based on haplotype frequencies (Raymond and Rousset. 1995) was accomplished using Arlequin 3.0 (Excoffier *et al.*, 2005).

The pairwise mismatch distribution for the entire populations was generated using Arlequin 3.0 (Excoffier *et al.*, 2005), and demographic parameters θ_0 and θ_1 (and their associated SSD *P* values) were estimated using a parametric bootstrap (Rogers and Harpending, 1992; Rogers, 1995). Harpending's (1994) raggedness index was computed for mismatch distribution, and its significance tested with 1000 replicates of bootstrap in Arlequin 3.0 (Excoffier *et al.*, 2005). *Snn* statistic developed by Hudson (2000) and implemented in DnaSP 4.1 (Rozas *et al.*, 2003) was applied to evaluate the proportion of nearest-neighbor haplotypes in the area shared by both species. We also used the statistics *D* (Tajima (1989) and *Fs* (Fu, 1997) implemented in the Arlequin 3.0 and in DnaSP 4.1 to infer deviations from neutrality of *L. purpureus* and *L. campechanus* populations.

Ninety-three *L. purpureus* from northern coastal waters of Brazil (37 from Amapá, 30 from Pará and 26 from Maranhão) were compared with 27 *L. campechanus* previously studied by Garber (2001). Two sequences of *Lutjanus synagris* from Pará were used as outgroup in the phylogenetic analysis. A total of 143 variant sites were found of which 99 were informative for parsimony (82 with two and 15 with three variants) and 43 were singletons. A total of 89 haplotypes were observed of which 35 were from Amapá, 29 from Pará and 25 from Maranhão populations. The highest number of absolute differences among haplotypes was 24, and the total haplotype diversity was 0.997. The USA populations showed the highest levels of haplotype diversity (1.0), probably as a consequence of the small sample sizes. Nucleotide diversity levels ranged from 0.014 in Louisiana to 0.027 in Pará and Maranhão populations (Table 1). North and South Atlantic populations have high haplotype and nucleotide diversities, which according to Grant and Bowen (1998) is suggestive of stable populations with long evolutionary history or secondary contact between differentiated lineages.

Both Tajima's *D* and Fu's *Fs* tests were implemented to test deviations from neutrality, however, demographic

Table 1 - Measures of mitochondrial DNA haplotype (*h*) and nucleotide (π) diversity, and neutrality tests observed based on the D-loop region of red snappers from six localities of the Western Atlantic.

Localities	N	H	h ± sd	π ± *sd*	*D* Tajima	*Fs* Fu
Mississippi (Garber, 2001)	13	13	1.000 ± 0.063	0.019 ± 0.039	-1.605^{ns}	-7.329**
Louisiana (Garber, 2001)	6	6	1.000 ± 0.096	0.014 ± 0.014	-1.124^{ns}	-1.897 ^{ns}
Florida (Garber, 2001)	8	8	1.000 ± 0.063	0.023 ± 0.034	-1.296^{ns}	-2.394*
North Atlantic (USA)	27	26	0.997 ± 0.011	0.018 ± 0.002	-1.912*	-21.55**
Amapá	37	35	0.997 ± 0.007	0.025 ± 0.001	-1.486^{ns}	-24.60**
Pará	30	29	0.998 ± 0.009	0.027 ± 0.001	-1.083^{ns}	-20.10**
Maranhão	26	25	0.997 ± 0.012	0.027 ± 0.002	-1.442^{ns}	-15.19**
South Atlantic (Brazil)	93	83	0.997 ± 0.010	0.026 ± 0.001	-1.677^{ns}	-99.00**
All	120	105	0.997 ± 0.002	0.025 ± 0.001	-1.862*	-34.26**

N = sample size; H = number of haplotypes; *h* = haplotype diversity; π = nucleotide diversity; sd = standard deviation; ns = no significant; *D* = Tajima statistic (Tajima, 1989); *Fs* = Fu statistic (Fu, 1997).
*p < 5%; **p < 1%.

interpretations are also valid in situations where selective neutrality cannot be discounted, such as in the mitochondrial control region (Rand, 1996; Hartl and Clark, 1997). Tajima's *D* was negative and significant only for North Atlantic populations as a whole, but not for individual populations. On the other hand, Tajima's D was negative but not significant for the three South Atlantic populations. As it is well known the expected value of Tajima's *D* statistic is equal to zero under the null hypothesis and negative values reflect an excess of low-frequency variants in the population, consistent with positive selection or expansion (Tajima, 1989; Fu and Li, 1993). However, Fu's *Fs* statistic test of neutrality tends be negative when there is an excess of recent mutations (population growth) as seems to be the case in the present study for all populations, except for the small sample (N = 6) of Louisiana. The apparent conflict between *D* and *Fs* statistics could be due different sensitivity of these tests to sample size effect.

The phylogenetic tree showed no evidence of separation between *L. campechanus* and *L. purpureus* (Figure 1), showing an unresolved polytomy. AMOVA also shows that North and South Atlantic red snappers are not significantly different (F/Φ_{CT} = 0.1379, ns), with almost 90% of the variance being found within populations (significant F/Φ_{ST} value of 0.1311) (Table 2). The exact test of sample differentiation based on haplotype frequencies (Raymond and Rousset, 1995) also showed no genetic differentiation between populations, and similar conclusions were reached based on the very sensitive *Snn* test (Hudson. 2000), which showed a value of 0.297 when all sub-samples of *L. campechanus* and *L. purpureus* were compared. Interestingly, mismatch distribution built for the entire group of *Lutjanus* showed a unimodal pattern (Figure 2) which is usually interpreted as representing populations that have passed through recent demographic expansion (Slatkin and Hudson, 1991; Rogers and Harpening, 1992) or through a

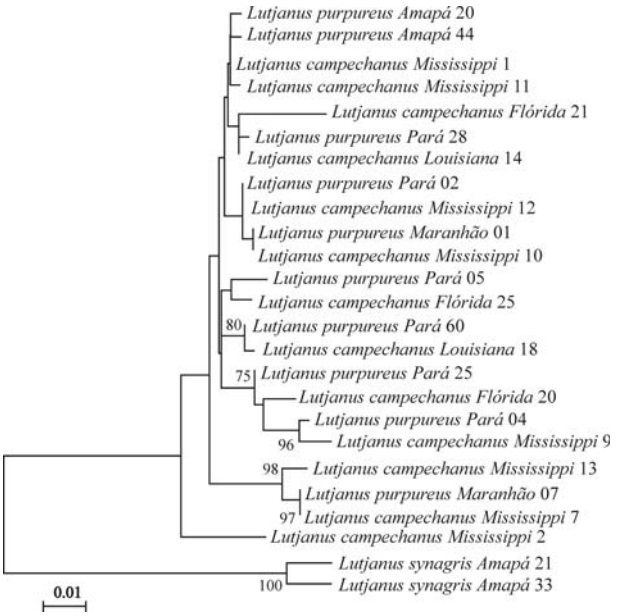

Figure 1 - Neighbor joining tree of *L. purpureus* and *L. campechanus* populations from the West Atlantic based on the control region of mitochondrial DNA. Numbers in the branches are bootstrap values obtained with 1000 pseudoreplicates. Bootstrap values shown in the tree are = 75%. The numbers beside each location are the registration of the sample in the laboratory.

range expansion with high levels of migration between neighboring demes (Ray *et al.* 2003, Excoffier 2004). Considering these results, the hypothesis of a single species of red snappers cannot be rejected.

As morphological and the present mitochondrial data are not able to discriminate between the two Atlantic red snappers species, the most plausible and parsimonious hypothesis would be that *Lutjanus* from the North and South Atlantic Ocean represent slightly different populations of a single species with a large geographical distribution. This would be in agreement with the suggestion of Cervigón

Table 2 - Analysis of Molecular Variance (AMOVA) for populations of *L. campechanus* and *L. purpureus* from six localities based on mitochondrial D-loop sequences.

Source of variation	Df	Variance components	Percentage of variation	F/Φ statistic	p
North x South populations					
Among groups	1	0.81019 Va	13.79	$F/\Phi_{CT} = 0.1379$	NS
Among populations	4	-0.04006 Vb	-0.68	$F/\Phi_{SC} = -0.0079$	NS
Within populations	114	5.10436 Vc	86.89	$F/\Phi_{ST} = 0.1311$	< 0.001
All	119	5.87449			
All populations					
Among populations	5	0.32368 Va	5.96	$F/\Phi_{ST} = 0.0596$	< 0.001
Within populations	114	5.10436 Vb	94.04		
All	119	5.42804			

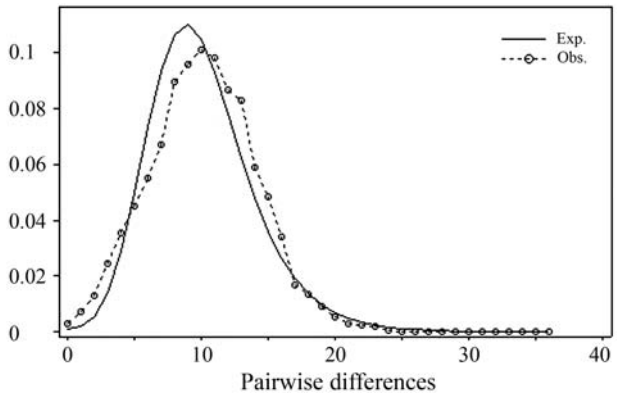

Figure 2 - Mismatch distribution based on 120 D-loop sequences of *L. purpureus* and *L. campechanus* from six localities of the West Atlantic. The solid line is the observed and the dotted line is the expected distribution of the pairwise differences.

(1993). However, considering the economic importance of this overexploited fish resource, and all legal questions related to stock management ,as well as the mislabelling issue raised by Marko *et a*l (2004), any decision about their taxonomic status should be well supported by additional genetic data, including the analysis of more populations along the entire distribution range of *L. purpureus/campechanus*, as well as additional representatives of the Lutjanidae family.

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