High Levels of Genetic Connectivity among Populations of Yellowtail Snapper, *Ocyurus chrysurus* (Lutjanidae – Perciformes), in the Western South Atlantic Revealed through Multilocus Analysis

Raimundo da Silva[1], Ivana Veneza[1], Iracilda Sampaio[2], Juliana Araripe[2], Horacio Schneider[2], Grazielle Gomes[1,2]*

1 Laboratório de Genética Aplicada, Instituto de Estudos Costeiros, Campus Bragança—Universidade Federal do Pará, Bragança, Pará, Brasil, 2 Laboratório de Genética e Biologia Molecular, Instituto de Estudos Costeiros, Campus Bragança—Universidade Federal do Pará, Bragança, Pará, Brasil

* grazielle@ufpa.br

Abstract

In the present study, five loci (mitochondrial and nuclear) were sequenced to determine the genetic diversity, population structure, and demographic history of populations of the yellowtail snapper, *Ocyurus chrysurus*, found along the coast of the western South Atlantic. *O. chrysurus* is a lutjanid species that is commonly associated with coral reefs and exhibits an ample geographic distribution, and it can therefore be considered a good model for the investigation of phylogeographic patterns and genetic connectivity in marine environments. The results reflected a marked congruence between the mitochondrial and nuclear markers as well as intense gene flow among the analyzed populations, which represent a single genetic stock along the entire coast of Brazil between the states of Pará and Espírito Santo. Our data also showed high levels of genetic diversity in the species (mainly mtDNA), as well a major historic population expansion, which most likely coincided with the sea level oscillations at the end of the Pleistocene. In addition, this species is intensively exploited by commercial fisheries, and data on the genetic structure of its populations will be essential for the development of effective conservation and management plans.

Introduction

Preservation of the biological diversity of any ecosystem is essential for evaluation of the distribution and connectivity of its populations [1] and the factors that determine these patterns. Considering the marine environment, opportunities for isolation to occur between populations are rare [2–4]. Many marine fish species tend to present a high degree of genetic connectivity, despite being distributed over thousands of kilometers of ocean, although this is often attributed to the

intense mixing of individuals during the initial phases of development [2,5,6]. In fact, genetic connectivity has often been associated with the duration of the pelagic larval phase (PLD) [7], although a number of studies have shown that there is not always a clear relationship between the duration of this phase and the genetic homogeneity of populations [8,9].

The yellowtail snapper, *Ocyurus chrysurus* (Bloch 1790), is a lutjanid fish found in tropical and subtropical coastal regions, where it is generally associated with sandy bottoms and coral reefs [10]. This species occurs in the western Atlantic between Florida (USA) and southeastern Brazil [10,11]. Similar to other lutjanid species, *O. chrysurus* exhibits a pelagic larval development period of approximately 30 days [12]. Following settlement of pelagic larvae, some studies indicate that the movements of the juveniles and adults of this species are somewhat limited [13,14], which may restrict gene flow among populations. A recent study [15] that included specimens from the Florida coast and the Caribbean and analyzed both mitochondrial (ND4 gene) and nuclear data (microsatellites) found that gene flow among populations was restricted and identified four distinct stocks of *O. chrysurus* in the region, despite not finding high levels of genetic divergence between populations. These results were attributed to a set of factors, particularly the influence of ocean currents and limitations on the movement of the post-larvae and adults [15].

Additional studies have provided evidence of the sub-structuring of yellowtail snapper stocks in the western Atlantic, including the Caribbean [16]. Vasconcellos et al. [16] analyzed populations from the coast of Brazil (Ceará, Pernambuco, Bahia, and Espírito Santo) and the Caribbean (Belize) based on morphometric data, allozymes, and sequences of mitochondrial DNA (Control Region) and identified a single Brazilian stock, revealing significant levels of genetic sub-structuring between populations from Belize and Brazil.

In spite of the economic and ecological relevance of this species as a fishery resource, Vasconcellos et al. [16] conducted the only genetic study of the Brazilian populations of *O. chrysurus* reported to date. Additionally, there was a large gap between the northernmost Brazilian population examined by these authors, in the state of Ceará, and Belize. In others words, the northern limit of the Brazilian stock—or how many stocks exist—remained unclear, considering the enormous extent of the northern sector of this country's coastline. Distinct stocks display independent evolutionary dynamics and can respond in different ways to intense fishing pressure [17]. Therefore, reliable information on these stocks is essential for fishery management and the conservation of the species.

Phylogeographic research in the western Atlantic (e.g., Brazil and Caribbean) has revealed a lack of effective barriers to gene flow in some fish species, such as two demersal lutjanids, the Southern and Northern red snappers, *Lutjanus purpureus* [5] and *Lutjanus campechanus* [6], respectively. Given the evidence of population sub-structuring between the Caribbean and Brazilian coast in the yellowtail snapper and the sampling gap in the northern Brazil in previous studies (resolved through the inclusion of samples from Maranhão and Pará) [16], the present study assessed the genetic connectivity among *O. chrysurus* populations distributed along more than 3,000 km of the coast of the western South Atlantic in Brazil (i. g. representing most of the species distribution in the Brazilian coast) and provides robust data on the population structure, genetic variability, and demographic history of this species.

Materials and Methods

Sampling

Specimens from a total of 170 *O. chrysurus* adults were collected from eight localities on the coast of Brazil, between 2007 and 2012, in the states of Pará, Maranhão, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Bahia, and Espírito Santo (Fig. 1). The tissues were obtained
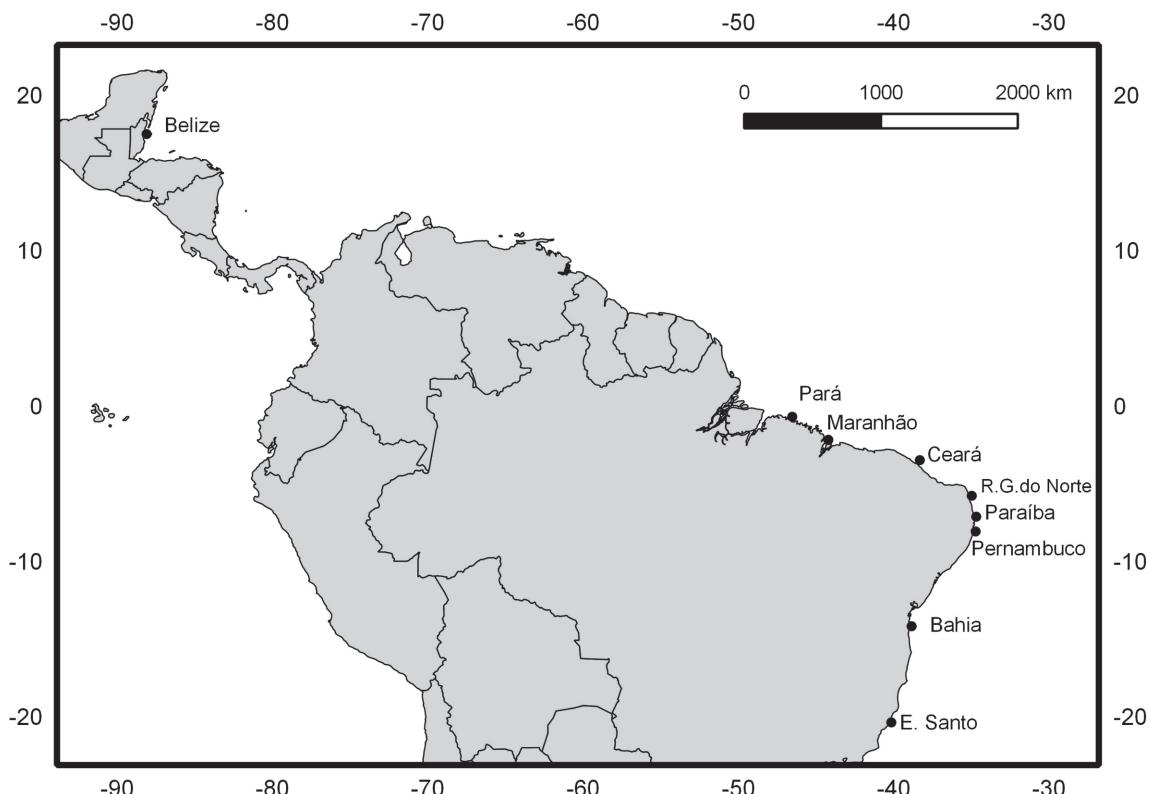
Fig 1. Map of collection locations for the present study. Distribution of the localities on the coast of Brazil from which the *Ocyurus chrysurus* specimens were collected for the present study, as well as location of Belize, previously sampled by Vasconcellos et al.[16].

doi:10.1371/journal.pone.0122173.g001

from commercial fishing ports (obtained from the near-shore artisanal fishery) and the fishing with rafts, performed by local fisherman. The specimens were identified based on the specific literature [10,18].

Ethics Statement

All specimens were obtained from dead individuals, procured through direct purchase from commercial landings in the localities mentioned above. *Ocyurus chrysurus* is not endangered or protected along the Brazilian Coast. Therefore, there was no need to apply for a license for collection or approval by the Animal Ethics Committee. The specimens were transported with the authorization of the Brazilian Environment Ministry.

Laboratory procedures

In the laboratory, tissues samples (from the muscle, fin or tongue) were extracted from each specimen and frozen until analysis. All of the specimens were included in the Lutjanidae tissue bank of the Applied Genetics Laboratory at the Bragança, Campus of the Federal University of Pará (UFPA). Their genomic material was obtained using a phenol-chloroform and enzymatic extraction protocol and was precipitated with sodium acetate, isopropanol and ethanol [19].

For this study, we analyzed two mitochondrial markers, Cytochrome b (Cytb) and the control region (CR) [20,21], and three intragenic nuclear regions, Adenine Nucleotide Transporter—intron 1 (ANT-1), Growth Hormone—intron 5 (GH-5) and Insulin-Like Growth Factor 1—intron 2 (IGF-2) [22–24]. These segments were amplified using polymerase chain reaction (PCR)

([Table 1](#)). The reactions were run in a volume of 15 μL, which included approximately 100 ng of total DNA, 2.4 μL of dNTPs (1.25 mM), 1.5 μL of buffer (200 mM Tris-HCl- pH 8.0, 500 mM KCl), 0.6 μL of MgCl2 (50 mM), 0.6 μL of each primer (50 ng/μL), 0.1 μL of Taq DNA polymerase (5 U/μL), and ultrapure water added to complete the reaction volume.

The amplicons were purified with PEG 8000 (polyethylene glycol) following the protocol [25] and sequenced via the dideoxy-terminal method [26] using the reagents of the Big Dye kit (ABI Prism Dye Terminator Cycle Sequencing Reading Reaction—PE Applied Biosystems, Carlsbad, CA, USA). The precipitate was sequenced through capillary electrophoresis in an ABI 3500 automatic sequencer (Applied Biosystems).

Initially, only one of the strands of each genomic region was sequenced (see [Table 1](#)). When ambiguities were observed in the chromatograms, the sample was sequenced in both directions and/or twice in the same direction (especially in the intragenic regions) to avoid errors in the identification of heterozygous individuals. For the intron of Insulin-like Growth Factor (IGF), it was necessary to design an internal primer (IGF B-5'- CATTGATATTCCTGNTCGTTCA-3') to obtain sequences in both directions. All haplotypes were deposited in Genbank under the accession numbers KM596919 to KM507050, except for GH-5, because that fragments smaller than 200 bp are not accepted in Genbank (these sequences are listen in [S1 Supporting Information](#)).

Database

The DNA sequences were edited and aligned in BIOEDIT v. 7.1.3.0 [27]. In the case of the nuclear loci, the individual heterozygotes were detected when double peaks were observed at the same position in both directions in the chromatograms. Heterozygotic events caused by indels were diagnosed through visual analysis of the chromatograms, with the alleles being reconstructed in INDELLIGENT v. 1.2. (http://imperialis.inhs.illinois.edu/dmitriev/indel.asp) [28].

The gametic phase of each nuclear marker was defined using the PHASE algorithm [29], available in DNAsp v 5. 10.01 [30]. The runs consisted of 1,000 burn-in iterations and 1,000 principal iterations, with a thinning interval of 1. The algorithm was applied five times, with the fifth chain being ten times longer than the others. The haplotypes that returned a probability of less than 0.8 were excluded from the analyses.

For the nuclear data, the minimum number of recombination events was estimated via the Rm method [31], available in DNAsp v 5. 10.01 [30]. As the results of this analysis are strongly affected by homoplasy [32], the significance of the number of recombination events was

Table 1. Primers used in the present study for Brazilian *Ocyurus chrysurus*.

Marker	Primers	Reference	Sequence (5'-3')	Annealing
CR	Dloop-A*	Lee et al.[20]	TTCCACCTCTAACTCCCAAAGCTAG	57°C
	Dloop-G	Lee et al.[20]	CGTCGGATCCCATCTTCAGTGTTATGCTT	
Cyt b	FishCybF*	Sevilla et al. [21]	ACCACCGTTGTTATTCAACTACAAGAAC	54°C
	TrucCytbR	Sevilla et al. [21]	CCGACTTCCGGATTACAAGACCG	
IGF 2	FCmugilF*	Rodrigues-Filho [24]	GTTCACAGCGCCACACAGAC	64°C
	FCmugilR*	Rodrigues-Filho [24]	CTTGAAGGATGAATGACTATGTCCA	
GH 5	GH5F*	Hassan et al. [23]	AGGCCAATCAGGACGGAGC	57°C
	GH6R*	Hassan et al. [23]	TGCCACTGTCAGATAAGTCTCC	
ANT 1	ANTF1*	Jarman et al. [22]	TGCTTCGTNTACCCVCTKGACTTTGC	56°C
	ANTR1*	Jarman et al. [22]	CCAGACTGCATCATCATKCGRCGDC	

*Primers used for sequencing.

doi:10.1371/journal.pone.0122173.t001

evaluated using the Φ_W test [32], available in SPLITS TREE v. 4.12.6 [33]. Linkage disequilibrium was analyzed the EM algorithm in ARLEQUIN v. 3.5.1.3 [34], which was run five times, with 20,000 permutations.

Characterization of genetic diversity and levels of gene flow

Determination of the number of polymorphic sites and the identification of possible stop codons (in the case of codifying regions) were performed in MEGA 6 [35]. The identification, quantification, and distribution of the haplotypes were determined in DNAsp v 5. 10.01 [30]. The genetic variability of the populations was evaluated based on the haplotype (h) and nucleotide (π) diversity indices [36] obtained from ARLEQUIN v. 3.5.1.3 [34].

The haplotype network was generated using HAPLOVIEWER [37] based on a maximum parsimony tree produced in DNAPARS, available in the package PHYLIP v. 3. 6 [38], in accordance with Salzburger et al. [37].

The genetic homogeneity of the *O. chrysurus* populations was initially evaluated through an analysis of molecular variance (AMOVA) [39] for each marker individually and subsequently through a multilocus approach, both with 10,000 permutations. This analysis permitted partitioning of the results into within- and between-population variation. In addition, F_{st} values [40] were used to evaluate the gene flow between pairs of populations. These analyses were run in ARLEQUIN v. 3.5.1.3 [34], with a subsequent adjustment of the *p* values using the False Discovery Rate test [41]. To verify the existence of isolation by distance, Mantel tests were performed using a matrix of genetic ($Fst/(1-Fst)$) and geographic (km converted to Ln) distances [42]. Negative Fst values were expressed as zero. These analyses were conducted in IBDWS (http://ibdws.sdsu.edu/~ibdws) [43], with 10,000 permutations.

For comparison between Brazilian and Caribbean populations, the control region sequences used by Vasconcellos et al. [16] (accession numbers EF624354—EF624359; EF624361—EF624373) were included in the network, AMOVA, and pairwise Fst analyses as well the Mantel test.

Bayesian methods, using STRUCTURE v. 2.3.4 [44], were applied to assign individuals to populations. This procedure places individuals into K clusters, where K is chosen in advance but can be experimentally varied throughout independent runs. K values between 1 and 8 were tested, using a model with admixture and no locprior (i. e., only genetic data is used for the assignment of individuals to a given K). For this analysis, only nuclear data were employed. Each run consisted of 1,000,000 steps (burn-in = 10%, and each value of K was implemented 10 times). The number of K was inferred by comparing the mean values of Ln Prob obtained in Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/) [45].

Cluster analyses were also conducted in STRUCTURAMA [46]. For this analysis, the mitochondrial and nuclear data were grouped. The runs consisted of 2,000,000 generations, (burn-in = 20%). For the values of K, we employed the following distribution (K = expk (2)). The runs were summarized using the "showtogetherness" command.

To check the fit of the historical population dynamics to a model of exponential growth, we used a mismatch distribution [47] together with the SSD and raggedness indices. Mismatch analyses were conducted in DNAsp v 5 10 01 [30], rates of SSD and raggedness, were implemented in Arlequin v. 3.5.1.3 [34] based on 10, 000 permutations.

Historic fluctuations in the demography of *O. chrysurus* were visualized using a Bayesian Skyline Plot (BSP) [48] and an extended Bayesian Skyline Plot (EBSP) [49]. These procedures were run in BEAST v.1.7.4 [50], based on evolutionary models suggested by JMODELTEST 2.1.1 [51,52] (HKY for Cytb, IGF 2, GH 5, ANT 1 and HKY+ I + G for CR). The analyses were based on the strict molecular clock used for the teleost control region, with a substitution rate of 10% per million years [53,54].

Two runs were performed using different random seeds, including 200 million generations for each BSP run and 400 million for each EBSP run, with samples taken at intervals of 10,000 generations, 10% of which were discarded as burn-in. The convergence and mixing of the chains were inspected visually in TRACER v.1.5 [55]. The convergence and mixing were considered to be appropriate when all of the ESS values for each of the parameters analyzed were above 200.

Tajima's *D* [56] and Fu's *Fs* [57] were also calculated, given that in addition to the detection of possible deviations from neutrality, these values may be used to evaluate demographic patterns, such as population expansion. These analyses were run in ARLEQUIN v. 3.5.1.3 [34], with their statistical significance being assessed using 10,000 permutations.

Results

Mitochondrial DNA

A total of 602 base pairs (bp) was sequenced from the Control Region in 152 *O. chrysurus* specimens, and 645 bp of the Cytochrome b gene was sequenced in 170 specimens (Table 2). Considering the respective evolutionary rates of the two markers, similar patterns concerning their distribution and haplotype frequencies were observed in the different populations examined. In the CR, which includes 93 polymorphic sites, a total of 91 haplotypes were identified, the most common of which was shared by 27 specimens and was present at all localities except Bahia. All other CR haplotypes were either unique or occurred at low frequencies and were distinguished by a small number of mutations. Only 12 polymorphic sites were found in Cytochrome b; however, a total of 12 haplotypes were identified, two of which were very common, being shared by 74 (44%) and 67 (39%) of the specimens and being found in all of the populations analyzed.

The indices of haplotypic diversity were high in the CR (h = 0.963±0.010) and lower for Cytb (h = 0.653±0.002), and the same pattern was observed in the case of nucleotide diversity, with π = 1.7% for the CR, but only π = 0.15% for Cytb (Table 2). AMOVA indicated that most of the variation in both markers occurs within populations, rather than between them, with low and non-significant Φ_{ST} values being obtained (Table 3), and this finding was further corroborated by the non-significant F_{st} values obtained in the pairwise comparisons between populations (Table 4). However, the comparison between the populations from the Brazilian coast and Belize revealed that approximately 30% of the variance is explained by differences between these two regions (Table 3). The Fst values between the Brazil and Belize populations were greater than 0.20 and were highly significant for all comparisons (Table 4), indicating particularly high differentiation between these stocks.

The genetic homogeneity of the populations from the Brazilian coast was also emphasized by the distribution of the haplotypes, given the lack of any clear geographic pattern in the network (Fig. 2). This genetic homogeneity over a broad geographic scale was further supported by the Mantel test, which rejected the scenario of isolation by distance (IBD), although when the comparisons included Belize there was some evidence in favor of a scenario with IBD, (Mantel test; r = 0.7354, p = 0.05) (S1 Fig.). The haplotypes identified in Belize were not shared by any locality analyzed along the Brazilian coast, supporting the significance of the Mantel test.

With regard to the neutrality of the data, the obtained *Fs* values were significant in some populations, or when the specimens were grouped in a single population for both mitochondrial markers (Table 2). The *D* values were not significant for any population (Table 2).

The Bayesian Skyline Plot (Fig. 3) indicated the historic occurrence of an increase in the effective size of the *O. chrysurus* populations, dated to the end of the Pleistocene. These results

Table 2. Genetic Diversity and statistics of neutrality for the Brazilian *Ocyurus chrysurus* populations analyzed in the present study.

Locus/Locality	N	Unique haplotypes	Nh	S	h (sd)	π (%)	Tajima's D	Fu's Fs
Control Region								
PA	30	16	26	50	0.983 (0.016)	1.79	−0.55 ns	−12.16 **
MA	11	7	8	36	0.890 (0.091)	1.56	−1.10 ns	0.10 ns
CE	31	17	26	61	0.982 (0.015)	2.01	−0.79 ns	−9.94 **
RN	22	11	18	50	0.956 (0.036)	1.77	−0.88 ns	−5.04 (0.029)
PB	21	7	17	51	0.966 (0.030)	1.90	−0.79 ns	−3.95 ns
PE	22	7	15	39	0.943 (0.035)	1.50	−0.61 ns	−2.42 ns
BA	8	3	6	26	0.928 (0.084)	1.93	0.83 ns	1.23 ns
ES	7	3	7	34	1 (0.076)	2.15	−0.38 ns	−1.12 ns
Total	**152**		**91**	**93**	**0.963 (0.010)**	**1.79**	**−1.11 ns**	**−24.22****
Cytochrome b								
PA	29	1	5	5	0.615 (0.052)	0.12	−1.04 ns	−1.29 ns
MA	16	2	7	7	0.791 (0.076)	0.21	−1.19 ns	−2.89 *
CE	31	1	6	6	0.707 (0.054)	0.18	−0.64 ns	−1.15 ns
RN	24	2	7	7	0.731 (0.064)	0.17	−1.23 ns	−2.69 (0.027)
PB	23	-	4	5	0.557 (0.083)	0.12	−1.22 ns	−0.40 ns
PE	25	1	4	4	0.616 (0.063)	0.14	−0.38 ns	0 ns
BA	10	-	3	2	0.711 (0.086)	0.13	0.83 ns	0.25 ns
ES	12	-	3	3	0.621 (0.103)	0.13	−0.04 ns	0.39 ns
Total	**170**		**12**	**12**	**0.653 (0.022)**	**0.15**	**−1.33 ns**	**−5.43 (0.024)**
IGF 2								
PA	22	4	12	16	0.811 (0.040)	1.43	1.95 ns	0.70 ns
MA	15	-	7	14	0.735 (0.054)	1.39	1.84 ns	3.40 ns
CE	27	4	14	14	0.808 (0.035)	1.39	2.36 ns	−0.01 ns
RN	21	1	11	15	0.815 (0.038)	1.44	2.25 ns	1.25 ns
PB	18	1	12	15	0.712 (0.069)	1.30	1.35 ns	−0.28 ns
PE	8	-	4	10	0.691 (0.073)	1.37	2.96 ns	5.11 ns
BA	8	2	8	14	0.891 (0.047)	1.37	1.08 ns	0.26 ns
ES	11	-	6	11	0.757 (0.074)	1.21	2.00 ns	2.80 ns
Total	**130**		**28**	**20**	**0.785 (0.017)**	**1.40**	**2.01 ns**	**−2.36 ns**
GH 5								
PA	26	-	5	4	0.451 (0.079)	0.45	−0.20 ns	−0.66 ns
MA	12	-	5	5	0.626 (0.093)	0.80	0.87 ns	0.01 ns
CE	33	-	4	3	0.489 (0.067)	0.52	0.89 ns	0.88 ns
RN	25	-	4	4	0.433 (0.079)	0.41	−0.41 ns	0.06 ns
PB	23	-	4	3	0.588 (0.068)	0.66	1.52 ns	1.26 ns
PE	13	1	5	5	0.624 (0.086)	0.84	0.36 ns	0.242 ns
BA	7	-	3	3	0.483 (0.142)	0.54	0.07 ns	0.78 ns
ES	11	-	4	3	0.463 (0.119)	0.55	0.50 ns	0.05 ns
Total	**150**		**8**	**6**	**0.510 (0.031)**	**0.57**	**0.10 ns**	**−0.96 ns**
ANT 1								
PA	26	-	2	1	0.110 (0.057)	0.03	−0.66 ns	−0.45 ns
MA	11	-	1	0	0.000 (0)	0.00	0 ns	0 ns
CE	33	-	2	1	0.192 (0.059)	0.06	−0.10 ns	0.35 ns
RN	24	-	2	1	0.119 (0.061)	0.03	−0.63 ns	−0.38 ns
PB	21	-	2	1	0.135 (0.068)	0.04	−0.58 ns	−0.26 ns

(Continued)

Table 2. (*Continued*)

Locus/Locality	N	Unique haplotypes	Nh	S	h (sd)	π (%)	Tajima's D	Fu's Fs
PE	7	-	2	1	0.142 (0.118)	0.04	−1.15 [ns]	−0.59 [ns]
BA	8	-	1	0	0.000 (0)	0.00	0 [ns]	0 [ns]
ES	12	-	1	0	0.000 (0)	0.00	0 [ns]	0 [ns]
Total	**142**		**2**	**1**	**0.112 (0.024)**	**0.03**	**−0.28[ns]**	**−0.00007[ns]**

Acronyms: N = number of specimens, Nh = number of haplotypes, S = number of segregating sites, h = haplotype diversity, π = nucleotide diversity.

*p< 0.05 (for Fs < 0.02)

**p< 0.01; ns = not significant; PA: Pará; MA: Maranhão; CE: Ceará; RN: Rio Grande do Norte; PB: Paraíba; PE: Pernambuco; BA: Bahia; Espírito Santo.

are consistent with a process of historical expansion of yellowtail snapper populations, as indicated by significant negative *Fs* values [57] and by adjusting the mismatch distributions to model population growth ([Fig. 4]).

Nuclear DNA

A fragment of 1,048 bp was sequenced for the nuclear regions, 552 bp of which corresponded to intron 2 of the Insulin-like Growth Factor (IGF) gene, which was sequenced in 130 specimens (13 individuals were excluded from the analysis due to the low *a posteriori* values recorded for their haplotypes). For this intron, an indel region of approximately 140 bp was identified. In the case of the Adenine Nucleotide Transporter (ANT) gene, 320 bp of intron 1 (together with a portion of the exons) was sequenced in 142 specimens, and 176 bp of intron 5 (together with a portion of the exon) of the Growth Hormone (GH) gene was sequenced in 150 specimens. The Φw test demonstrated the existence of recombination events only in IGF 2

Table 3. Analysis of Molecular Variance for the Brazilian *O. chrysurus* populations.

C R	Variance	Variation (%)	Φ
Among groups (Brazil x Belize)	2.70579	32.92262	0.32923**
Among groups/ Within groups	−0.07523	−0.91533	−0.01365
Within of populations	5.58807	67.99271	0.32007
CR			
Among populations	−0.06248	−1.15973	−0.01160[ns]
Within of populations	5.44961	101.15973	
Cyt b			
Among populations	−0.01012	−2.08757	−0.02088 [ns]
Within of populations	0.49468	102.08757	
nuDNA[1]			
Among populations	−0.00912	−1.32	−0.01324 [ns]
Within of populations	0.69809	101.32	

Analysis of Molecular Variance (AMOVA) for the Brazilian *O. chrysurus* populations analyzed in the present study, for control Region (Brazil/ Belize), Control Region (only Brazil), Cytochrome b, and nuDNA (intragenic markers –IGF 2; GH 5; ANT 1).

**- p< 0.01; ns = not significant

1 Due to the similar pattern obtained for each marker individually, we chose to show only the of the results analysis multiloci.

Table 4. Matrix of pairwise Fst values for the Brazilian populations of *O. chrysurus*.

CR

	BE	PA	MA	CE	RN	PB	PE	BA	ES
BE	-								
PA	0.295**	-							
MA	0.319**	−0.008 ns	-						
CE	0.269**	−0.009 ns	−0.014ns	-					
RN	0.288**	−0.020 ns	−0.035ns	−0.025 ns	-				
PB	0.272**	−0.022 ns	−0.014ns	−0.016 ns	−0.025 ns	-			
PE	0.325**	−0.018 ns	−0.030ns	−0.007 ns	−0.024 ns	−0.016ns	-		
BA	0.203**	−0.002ns	0.066ns	0.001ns	0.0001ns	0.005 ns	0.035ns	-	
ES	0.258**	0.014ns	0.008ns	0.0008ns	0.009ns	−0.026ns	0.016ns	0.077ns	-

Cyt b

	PA	MA	CE	RN	PB	PE	BA	ES
PA	-							
MA	−0.023ns	-						
CE	−0.012ns	−0.030ns	-					
RN	−0.017ns	−0.030ns	−0.032ns	-				
PB	−0.008ns	−0.009ns	−0.021ns	−0.027ns	-			
PE	−0.015ns	−0.034ns	−0.006ns	−0.008ns	0.021ns	-		
BA	−0.026ns	−0.054ns	−0.051ns	−0.060ns	−0.024ns	−0.030ns	-	
ES	−0.051ns	−0.044ns	−0.037ns	−0.039ns	−0.041ns	−0.018ns	−0.030ns	-

nuDNA

	PA	MA	CE	RN	PB	PE	BA	ES
PA	-							
MA	−0.018ns	-						
CE	−0.015ns	−0.027ns	-					
RN	−0.015ns	−0.019ns	−0.015ns	-				
PB	−0.010ns	0.004 ns	0.003 ns	0.0001ns	-			
PE	−0.030ns	−0.030ns	−0.017ns	−0.019 ns	−0.023ns	-		
BA	−0.013ns	−0.034ns	−0.023ns	−0.023 ns	0.013 ns	−0.019ns	-	
ES	−0.004ns	−0.028ns	−0.005ns	−0.002 ns	0.047 ns	−0.012ns	−0.012*ns	-

Matrix of pairwise Fst values for the Brazilian populations of *O. chrysurus* analyzed in the present study, for control Region, Cytochrome b, and nuDNA (intragenic markers—IGF 2; GH 5; ANT 1). Acronyms: BE- Belize, PA- Pará, MA- Maranhão, CE- Ceará, RN- Rio Grande do Norte, PB- Paraíba, PE- Pernambuco, BA- Bahia, ES- Espírito Santo

****- p = 0

*ns = -p<0.05, however non-significant after FDR

ns- Not significant

1- Due to the similar pattern obtained for each marker individually, we chose to show only the of the results analysis multiloci.

(Rm = 8; p = 0.03). When the indel region was removed, recombination was not detected (p = 0. 25), and all of the analyses for this marker were performed excluding this region.

The highest diversity was recorded in the IGF 2 sequences (h = 0.785 ± 0.017; π = 1.4%), with intermediate values (h = 0.510±0.031; π = 0.57%) being recorded for GH 5 and considerably lower values being obtained for ANT 1 (h = 0.112±0.024; π = 0.03%) ([Table 2]).

The results of the AMOVA revealed that most of the genetic variance was present within, rather than between the populations, which is consistent with high levels of genetic similarity between populations ([Table 3]). This finding was reinforced by the frequency and distribution
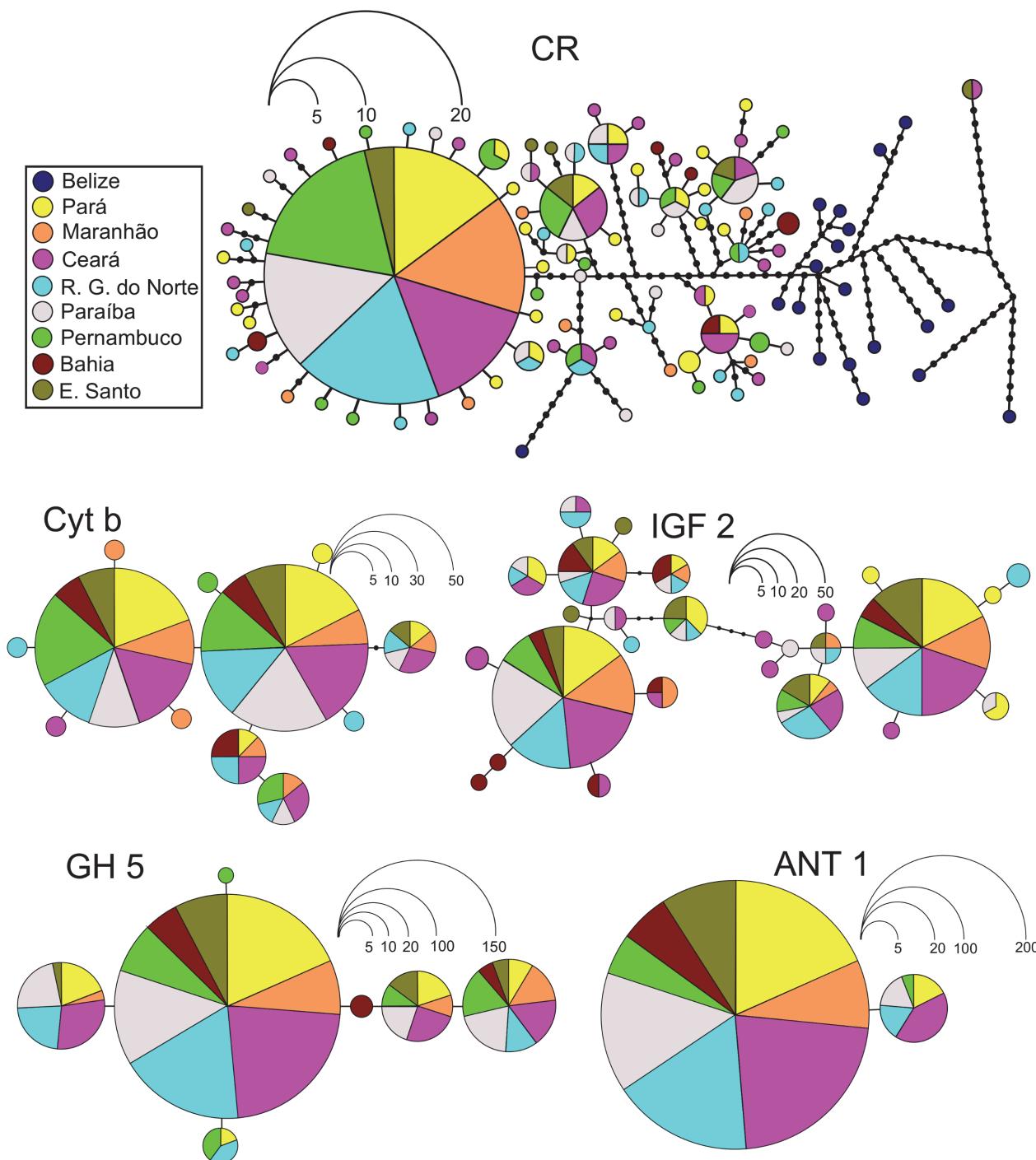
Fig 2. Genetic relationships among haplotypes found in the Brazilian *Ocyurus chrysurus* populations. Estimated by the maximum parsimony method, for the sequences of the Control Region, Cytochrome b, IGF 2, GH 5, and ANT. Each haplotype is represented by a circle, and the frequency of each haplotype proportional to the scale shown. Colors refer to the origin of each sample analyzed.

doi:10.1371/journal.pone.0122173.g002

of the identified haplotypes (Fig. 2) as well as by non-significant values of the pairwise Fst (Table 4) and Mantel tests (S1 Fig.). Likewise, the Bayesian clustering analysis showed that a scenario with only one group is the most likely (S2 and S3 Figs.).
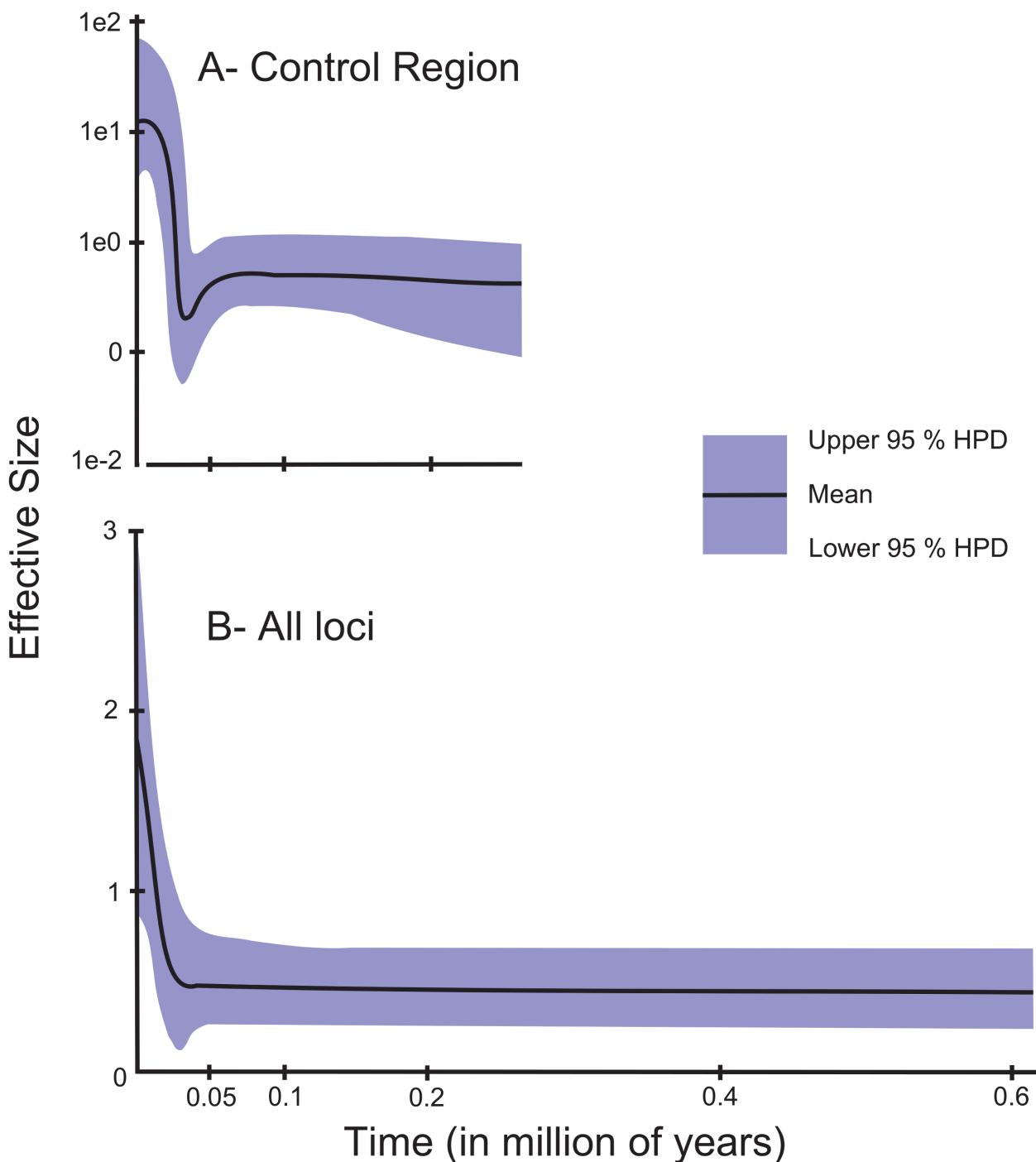
Fig 3. Skyline plots for Brazilian *Ocyurus chrysurus*. A. Based on the Control Region sequences (Bayesian Skyline plot). B. Extended Bayesian Skyline plot for the Brazilian *Ocyurus chrysurus* populations. Both are based on a mutation rate of 10% per million years (between lineages).

doi:10.1371/journal.pone.0122173.g003

With regard to the neutrality indices (Table 2), the obtained values were negative and not significant. The mismatch distributions were unimodal for all markers, except for IGF 2, which presented a clearly bimodal distribution (Fig. 4). This pattern can be observed in populations that have experienced long intervals with stable sizes or that have undergone a subtle decrease
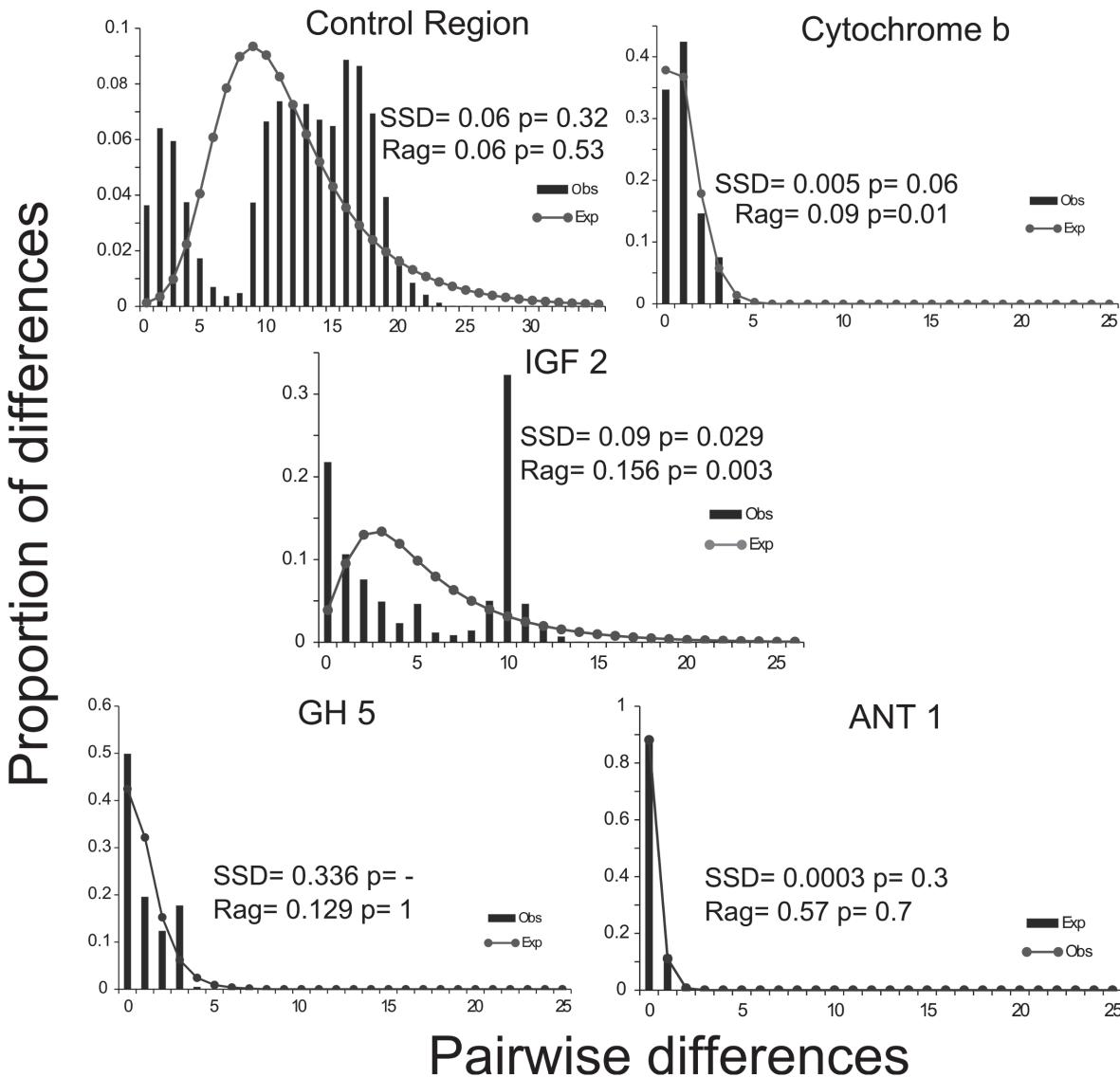
Fig 4. Distribution of the pairwise differences between haplotypes for *Ocyurus chrysurus* from the coast of Brazil. On the y axis the number of proportional differences is presented. X-axis represents the number of differences between pairs of sequences. Bars correspond to the observed values, and the dotted lines represent the proposed model for exponential growth.

doi:10.1371/journal.pone.0122173.g004

in their effective size, followed by an event of expansion of range, or in populations who have experienced a weak bottleneck [58]. The EBSP analysis indicated the occurrence of an increase in the effective size of the *O. chrysurus* populations (Fig. 3), supporting the results of the BSP for the mitochondrial Control Region.

Discussion

Genetic structure in the yellowtail snapper

The pattern of genetic homogeneity observed in *O. chrysurus* in the present study is similar to that found in other lutjanids, such as *Lutjanus kasmira* [59], *Pristipomoides filamentosus* [60], *L. campechanus* [6], and *L. purpureus* [5]. All of these species exhibit a high dispersal capacity during at least one phase of their life cycle. However, a pattern of significant genetic structure

has been observed for other species of this Family, such as *Lutjanus erythropterus* [53], *Lutjanus fulvus* [59], and *Lutjanus synagris* [61].

Differences in larval behavior as well as the characteristics of the environment where these species live may explain these discordant patterns of genetic connectivity and appear to directly influence the evolutionary history of these fish. However, it is important to note that *O. chrysurus* is not genetically homogeneous throughout the whole of its geographic distribution. Vasconcellos et al. [16] found marked differences between the Caribbean and Brazilian populations of *O. chrysurus*, while Saillant et al. [15] detected genetic sub-structuring in the populations of these species located in neighboring areas of the Caribbean and Gulf of Mexico. In general, these data are consistent with the existence of effective barriers in the ocean between northern Brazil and the Caribbean, (e.g., circulation patterns and ocean currents), as identified in previous phylogeographic studies [62–64], as well as between the Caribbean and the Gulf of Mexico [15,65]. Nevertheless, regarding genetic differentiation involving Brazilian populations, our results demonstrate that the model of isolation by distance cannot be discarded.

Juvenile and adult yellowtail snappers tend to remain within their area of settlement over the course of their lives [13,14]. This characteristic, in addition to specific habitat features, such as the pattern of currents, may account for the genetic differentiation observed in previous studies, with limited gene flow occurring between populations in some areas [15].

However, the larvae of *O. chrysurus* display a strong swimming ability [66], and they are most likely able to travel long distances, which could lead to intensive mixture of individuals. Moreover, these larvae present a planktonic phase of approximately one month and an offshore distribution, and during this larval stage, they preferably inhabit surface waters, which are more subject to the influence of ocean currents [12]. The dynamics of ocean surface circulation in the south of the western Atlantic region is primarily a result of bifurcation of the South Equatorial current [67]. Some studies, however, demonstrate that the bifurcation of the south equatorial current is not an effective barrier to dispersal for many marine taxa [68,69], furthermore, the main currents parallel to the Brazilian coast (i.e. Brazil Current / North Brazil Current) show slight seasonal changes in direction (see http://www.aoml.noaa.gov/phod/graphics/dacdata/seasonal_brazil.gif) that could allow some connectivity between the locations analyzed. Thus, all of these features are consistent with high genetic similarity along the Brazilian coast (coast Pará to Espírito Santo), as demonstrated in this study, and they may be the main factors regulating the genetic connectivity between the Brazilian populations of *O. chrysurus*, as observed in another lutjanid, *L. purpureus* [5].

Genetic Diversity

The results of the present analysis revealed high levels of genetic variation in the investigated yellowtail snapper populations, especially in the mitochondrial markers, which presented a large number of haplotypes, similar to that recorded for *L. purpureus* [5] and *L. campechanus* [6], with similar genetic variability demonstrated between localities, over a distance of approximately 3,000 km along the Brazilian coast. This outcome would be expected for populations linked by intense gene flow, a scenario commonly observed in a number of marine species [70]. The high indices of genetic diversity recorded in the present study, especially for the mitochondrial markers, and particularly the Control Region, are a common feature of marine teleosts, including lutjanids [5,6,53,71,72], and have been recorded previously in *O. chrysurus* [16].

This high genetic variability cannot be interpreted as an absence of an impact of fishing on these populations because the commercial exploitation of this species is relatively recent (approximately 50 years and less than 20 generations). Examples from the literature reveal several situations where overfishing apparently shows no direct relationship with genetic diversity

indices. For example, Hoarau et al. [73] did not report decreased levels of genetic variability in microsatellite markers in a temporal analysis of *Pleuronectes platissa* covering almost 100 years, even though this species has been heavily exploited since the XIX century. Additionally, Pinsky & Palumbi [74] recently performed a meta-analysis involving hundreds of fish species and observed high levels of genetic diversity for snappers of the genus *Lutjanus*, even for species that are considered to be overfished.

Distinct levels of genetic diversity were observed at the three nuclear loci analyzed in the present study. The most variable nuclear locus was the intron of the IGF 2 gene, which presented a degree of polymorphism comparable to that observed in the mitochondrial Control Region, indicating that it is a potentially useful marker for population-level and phylogeographic studies in lutjanids, as observed for *Centropomus* [75].

Demographic History

The yellowtail snapper inhabits coastal waters [10] and is therefore relatively susceptible to sea level oscillations [76]. During the last glacial maximum, for example, approximately 90% of the present-day continental shelf of the Caribbean region was above sea level, due to a decrease in the sea level of 120 meters [77]. This situation almost certainly led to the extinction of species or lineages, as well as contraction processes and population expansion [78, 79,80].

The temperature fluctuations that occurred in this period may have had a great influence on the historical demography of a number of marine species from this region. For example, Rocha et al. [81] lists temperature fluctuations that occurred in the Atlantic (see Sachs et al. [82]) as one of the events responsible for population growth in species of the *Chromis* genus. *O. chrysurus* generally prefers water at temperatures between 24–30°C, with temperatures above 34°C being lethal for this species [83]. Thus, temperature changes beyond these thresholds could also lead to strong fluctuations in the effective size of *O. chrysurus* populations.

Historic events of population expansion are commonly identified in demographic studies on marine fish species. In the western Atlantic, events of this type have been recorded in *Cynoscion guatucupa* [84] and *Chromis multilineata* [81] as well as in lutjanids, such as the snappers *L. purpureus* [5] and *L. campechanus* [6].

The results obtained for the *O. chrysurus* stocks analyzed in the present study clearly indicate population expansion along the Brazilian coast. This process is similar to that observed in Caribbean *O. chrysurus*, although Vasconcellos et al. [16] concluded that the Brazilian populations were demographically stable because all tests neutrality ("Fs" and "D") were unable to reject a neutral model of evolution. In this case, it is possible that the discrepancies in relation to the previous study are related to intrinsically different sensitivities of the applied neutrality tests. Finally, *Fs* test, was significant only for the total population for the mitochondrial markers examined in the present study. Fu's test shows greater statistical power and sensitivity for detecting events of geographical expansion [57] and is therefore more suitable for supporting the expansion scenario indicated by the mitochondrial markers.

The expansion of the Brazilian populations of *O. chrysurus* is also clear from the BSP and EBSP analyses, which identified an abrupt increase in population size approximately 25,000 years ago, which would coincide with the last glacial maximum in this region (see Barreto et al.) [85]. In fact, the recent Pleistocene glaciation events are well documented in the marine environment [82,86], and the marked changes in water temperatures and sea levels almost certainly had great effects on the historical dynamics of the population size of *O. chrysurus* as well as other marine organisms

Final considerations

The present study is the most comprehensive investigation of the Brazilian populations of *Ocyurus chrysurus* undertaken to date. The results clearly revealed the existence of a single panmictic population along the Brazilian coast (*sensu* Selkoe et al.)[87], and as reported by Vasconcellos et al. [16], there is a high level of substructure between the Brazilian and Caribbean populations. However, it is worth mentioning that the absence of individuals sampled between Brazil and Belize makes it difficult to perform further analyses of the historical connectivity between populations in this geographic range.

In relation to the high levels of genetic diversity observed, mainly among mitochondrial markers, these findings cannot be interpreted as a lack of any impact of fisheries on these populations, given that the commercial exploitation of this species is relatively recent, and the markers used in the present study would not be appropriate for the detection of alterations on this time scale (see Gaither et al. [88]). Furthermore, the deterioration of genetic diversity due to overfishing is more clearly demonstrated in populations that exhibit a low effective size (e.g., a few hundred individuals) and low migration rates [74], which may not be the scenario for *Ocyurus chrysurus* along the Brazilian coast.

The data presented herein suggest that the populations of yellowtail snapper along the Brazilian coast may represent a single genetic stock, and if so, they should be managed as a unit. This conclusion has strong implications for the management of this species. For example, for various snappers, including *O. chrysurus*, the formation of shoals for spawning is reported to occur at a number of locations [89]. Thus, the protection of these areas should be given a higher priority because they apparently have a greater impact on the population structure over a vast area.

Further studies involving other classes of molecular markers (e.g., microsatellite markers, SNPs, adaptive loci) as well as studies tracking adults and larval dispersal should be conducted to obtain a better understanding of the structure of *O. chrysurus* populations.

Supporting Information

S1 Fig. Correlation between genetic distance and geographic distance between sample sites for *O. chrysurus*.
(TIF)

S2 Fig. Diagrams representing the structure of the K values (1–8). Above Mean Ln prob values ± standard deviation for each value of K. Each individual is represented by a bar. The height of the bar is proportional to the probability of the individual belonging to a given cluster.
(TIF)

S3 Fig. Matrix indicating the pair-wise probability of each individual belonging to the same group. Each individual analyzed is represented by a square, and that the probability values are represented by color (see scale). Numbers: 1- Pará, 2- Maranhão, 3- Ceará, 4- R. G. do Norte, 5- Paraíba, 6- Pernambuco, 7- Bahia, 8- Espírito Santo.
(TIF)

S1 Supporting Information. Codes and sequences of Growth hormone- Intron 5 utilized in the present study.
(XLS)

Author Contributions

Conceived and designed the experiments: RS IV JA GG. Performed the experiments: RS IV GG. Analyzed the data: RS GG HS IS. Contributed reagents/materials/analysis tools: IS HS GG. Wrote the paper: RS IS JA HS GG. Drafting the article or revising it critically for important intellectual content: GG JA IS. Final approval of the version to be published: GG JA HS.

References

1. Craig MT, Hastings PA, Pondella DJ II, Ross Robertson D, Rosales-Casián JA. Phylogeography of the flag cabrilla *Epinephelus labriformis* (Serranidae): implications for the biogeography of the Tropical Eastern Pacific and the early stages of speciation in a marine shore fish. J Biogeogr. 2006; 33: 969–979.

2. Palumbi SR. Genetic Divergence, Reproductive Isolation, and Marine Speciation. Annu Rev Ecol Syst. 1994; 25: 547–572.

3. Rocha LA, Robertson DR, Roman J, Bowen BW. Ecological speciation in tropical reef fishes. Proc Biol Sci. 2005; 272: 573–579. PMID: 15817431

4. Bernardi G. Speciation in fishes. Mol Ecol. 2013; 22: 5487–5502. doi: 10.1111/mec.12494 PMID: 24118417

5. Gomes G, Sampaio I, Schneider H. 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. An Acad Bras Cienc. 2012; 84: 979–999. PMID: 23207703

6. Garber AF, Tringali MD, Stuck KC. Population structure and variation in red snapper (*Lutjanus campechanus*) from the Gulf of Mexico and Atlantic coast of Florida as determined from mitochondrial DNA control region sequence. Mar Biotechnol. 2004; 6: 175–185. PMID: 14586768

7. Planes S. Biogeography and Larval Dispersal Inferred from Population Genetic Analysis. In: Sale P., editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. New York, USA: Academic Press; 2002. pp. 201–220.

8. Shulman MJ, Bermingham E. Early Life Histories, Ocean Currents, and the Population Genetics of Caribbean Reef Fishes. Evolution. 1995; 49: 897–910.

9. Luiz OJ, Madin JS, Robertson DR, Rocha LA, Wirtz P, Floeter SR. Ecological traits influencing range expansion across large oceanic dispersal barriers: insights from tropical Atlantic reef fishes. Proc Biol Sci. 2012; 279: 1033–1040. doi: 10.1098/rspb.2011.1525 PMID: 21920979

10. Allen G-R. Snappers of the World.An annotated and illustrated catalogue of Lutjanid species known to date. Vol 6. Rome: FAO; 1985.

11. Cervigón F. Los peces marinhos de Venezuela. 2a edição. Caracas, Venezuela: Fundacion Científica Los Roques; 1993.

12. Lindeman KC, Lee TN, Wilson WD, Claro R, Ault JS. Transport of Larvae Originating in Southwest Cuba and Dry Tortugas: Evidence for Retention in Grunts and Snappers. Proc 52nd Gulf Caribb Fish Inst. 2001; 52: 732–747.

13. Lindholm J, Kaufman L, Miller S, Wagschal A, Newville M. Movement of yellowtail snapper (Ocyurus chrysurus Block 1790) and black grouper (Mycteroperca bonaci Poey 1860) in the northern Florida Keys National Marine Sanctuary as determined by acoustic telemetry: 2005. Available: http://aquaticcommons.org/2324/1/lindholm.pdf.

14. Watson M, Munro J, Gell FR. Settlement, movement and early juvenile mortality of the yellowtail snapper *Ocyurus chrysurus*. Mar Ecol Prog Ser. 2002; 237: 247–256.

15. Saillant EA, Renshaw MA, Cummings NJ, Gold JR. Conservation genetics and management of yellowtail snapper, *Ocyurus chrysurus*, in the US Caribbean and South Florida. Fish Manag Ecol. 2012; 19: 1–12.

16. Vasconcellos AV, Vianna P, Paiva PC, Schama R, Solé-Cava A. Genetic and morphometric differences between yellowtail snapper (*Ocyurus chrysurus*, Lutjanidae) populations of the tropical West Atlantic. Genet Mol Biol. 2008; 31: 308–316.

17. Ovenden JR, Berry O, Welch DJ, Buckworth RC, Dichmont CM. Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. Fish Fish. 2013; doi: 10.1111/faf.12052.

18. Menezes NA, Figueiredo JL. Manual de Peixes Marinhos do Sudeste do Brasil.IV.Teleostei (3). São Paulo: Museu de Zoologia-Universidade de São Paulo; 1980.

19. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. 2nd ed. New York, USA: Cold Spring Harbor Laboratory Press; 1989.

20. Lee W-J, Conroy J, Howell WH, Kocher TD. Structure and evolution of Teleost mitochondrial control regions. J Mol Evol. 1995; 41: 54–66. PMID: 7608989

21. Sevilla RG, Diez A, Norén M, Mouchel O, Jérôme M, Verrez-Bagnis V. Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes. Mol Ecol Notes. 2007; 7: 730–734.

22. Jarman SN, Ward RD, Elliott NG. Oligonucleotide primers for PCR amplification of Coelomate introns. Mar Biotechnol. 2002; 4: 347–355. PMID: 14961246

23. Hassan M, Lemaire C, Fauvelot C, Bonhomme F. Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish. Mol Ecol Notes. 2002; 2: 334–340.

24. Rodrigues-Filho LFS. Identificação e Filogeografia de Tainhas do Gênero Mugil e avaliação do estado taxonômico das espécies *Mugil liza* Valenciennes, 1836 e *Mugil platanus* Günther, 1880. Ph. D. Thesis, Universidade Federal do Pará. 2011.

25. Paithankar KR, Prasad KS. Precipitation of DNA by polyethylene glycol and ethanol. Nucleic Acids Res. 1991; 19: 1346. PMID: 2030954

26. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Procedings Natl Acad Sci USA. 1977; 74: 5463–5467. PMID: 271968

27. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999; 41: 95–98.

28. Dmitriev DA, Rakitov RA. Decoding of superimposed races produced by direct sequencing of heterozygous indels. PLoS Comput Biol. 2008; 4: e1000113. doi: 10.1371/journal.pcbi.1000113 PMID: 18654614

29. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001; 68: 978–989. PMID: 11254454

30. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25: 1451–1452. doi: 10.1093/bioinformatics/btp187 PMID: 19346325

31. Hudson RR, Kaplan NL. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics. 1985; 111: 147–164. PMID: 4029609

32. Bruen TC, Philippe H, Bryant D. A simple and robust statistical test for detecting the presence of recombination. Genetics. 2006; 172: 2665–2681. PMID: 16489234

33. Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. Mol Biol Evol. 2006; 23: 254–267. PMID: 16221896

34. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010; 10: 564–567. doi: 10.1111/j.1755-0998.2010.02847.x PMID: 21565059

35. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30: 2725–2729. doi: 10.1093/molbev/mst197 PMID: 24132122

36. Nei M. Molecular Evolutionary Genetics. New York: Columbia Univ. Press; 1987.

37. Salzburger W, Ewing GB, von Haeseler A. The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. Mol Ecol. 2011; 20: 1952–1963. doi: 10.1111/j.1365-294X.2011.05066.x PMID: 21457168

38. Felsenstein J. PHYLIP—Phylogeny Inference Package (version 3.2). Cladistics. 1989; 5: 164–166.

39. Excoffier L, Smouse PE, Quattro JM. Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. Genetics. 1992; 131: 479–491. PMID: 1644282

40. Weir BS, Hill WG. Estimating F-Statistics. Annu Rev Genet. 2002; 36: 721–750. PMID: 12359738

41. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Ann Stat. 2001; 29: 1165–1188.

42. Rousset F. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation-by-distance. Genetics. 1997; 145: 1219–1228. PMID: 9093870

43. Jensen JL, Bohonak AJ, Kelley ST. Isolation by distance, web service. BMC Genet. 2005; 6. doi: 10.1186/1471-2156-6-13.

44. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155: 945–959. PMID: 10835412

45. Earl DA, VonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2011; 4: 359–361.

46. Huelsenbeck JP, Andolfatto P, Huelsenbeck ET. Structurama: bayesian inference of population structure. Evol Bioinforma. 2011; 7: 55–59. doi: 10.4137/EBO.S6761 PMID: 21698091

47. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol. 1992; 9: 552–569. PMID: 1316531

48. Drummond AJ, Rambaut A, Shapiro B, Pybus OG. Bayesian coalescent inference of past population dynamics from molecular sequences. Mol Biol Evol. 2005; 22: 1185–1192. PMID: 15703244

49. Heled J, Drummond AJ. Bayesian inference of population size history from multiple loci. BMC Evol Biol. 2008; 8: doi: 10.1186/1471–2148–8–289.

50. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012; 29: 1969–1973. doi: 10.1093/molbev/mss075 PMID: 22367748

51. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximun likelihood. Syst Biol. 2003; 52: 694–704.

52. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012; 9: 772. doi: 10.1038/nmeth.2109 PMID: 22847109

53. Zhang J, Cai Z, Huang L. Population genetic structure of crimson snapper *Lutjanus erythropterus* in East Asia, revealed by analysis of the mitochondrial control region. ICES J Mar Sci. 2006; 63: 693–704.

54. Avise JC. Phylogeography The History and Formation of species. Cambridge, MA: Havard University Press; 2000.

55. Rambaut A, Drummond AJ. Tracer v1. 5. 2009. Available: http://beast.bio.ed.ac.uk. Accessed 23 August 2013.

56. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989; 123: 585–595. PMID: 2513255

57. Fu Y-X. Statistical Tests of Neutrality of Mutations Against Population Growth, Hitchhiking and Background Selection. Genetics. 1997; 147: 915–925. PMID: 9335623

58. Ray N, Currat M, Excoffier L. Intra-Deme Molecular Diversity in Spatially Expanding Populations. Mol Biol Evol. 2003; 20: 76–86. PMID: 12519909

59. Gaither MR, Toonen RJ, Robertson DR, Planes S, Bowen BW. Genetic evaluation of marine biogeographical barriers: perspectives from two widespread Indo-Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). J Biogeogr. 2010; 37: 133–147.

60. Gaither MR, Jones SA, Kelley C, Newman SJ, Sorenson L, Bowen BW. High connectivity in the deep-water snapper *Pristipomoides filamentosus* (Lutjanidae) across the Indo-Pacific with isolation of the Hawaiian archipelago. PLoS One. 2011; 6: e28913. doi: 10.1371/journal.pone.0028913 PMID: 22216141

61. Karlsson S, Saillant E, Gold JR. Population structure and genetic variation of lane snapper (*Lutjanus synagris*) in the northern Gulf of Mexico. Mar Biol. 2009; 156: 1841–1855.

62. Rocha LA, Bass AL, Robertson DR, Bowen BW. Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). Mol Ecol. 2002; 11: 243–252. PMID: 11856425

63. Rocha LA. Patterns of distribution and processes of speciation in Brazilian reef fishes. J Biogeogr. 2003; 30: 1161–1171.

64. Floeter SR, Rocha LA, Robertson DR, Joyeux JC, Smith-Vaniz WF, Wirtz P, et al. Atlantic reef fish biogeography and evolution. J Biogeogr. 2008; 35: 22–47.

65. Gold JR, Saillant E, Cummings NJ, Renshaw MA. Genetic Divergence and Effective Size among Lane Snapper in U. S. Waters of the Western Atlantic Ocean. North Am J Fish Manag. 2011; 31: 209–223.

66. Hogan JD, Fisher R, Nolan C. Critical swimming speed of settlement-stage coral reef fishes from the Caribbean: a methodological and geographical comparison. Bull Mar Sci. 2007; 80: 219–231.

67. Peterson RG, Stramma L. Upper-level circulation in the South Atlantic Ocean. Prog Ocean. 1991; 26: 1–73.

68. Rodríguez-Rey GT, Solé-Cava AM, Lazoski C. Genetic homogeneity and historical expansions of the slipper lobster, *Scyllarides brasiliensis*, in the south-west Atlantic. Mar Freshw Res. 2014; 65: 59–69.

69. Wieman A. C, Berendzen PB, Hampton KR, Jang J, Hopkins MJ, Jurgenson J, et al. A panmictic fiddler crab from the coast of Brazil? Impact of divergent ocean currents and larval dispersal potential on genetic and morphological variation in *Uca maracoani*. Mar Biol. 2013; 161: 173–185.

70. DeWoody JA, Avise JC. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J Fish Biol. 2000; 56: 461–473.

71. Gomes G, Schneider H, Vallinoto M, Santos S, Orti G, Sampaio I. Can *Lutjanus purpureus* (South red snapper) be "legally" considered a red snapper (*Lutjanus campechanus*)? Genet Mol Biol. 2008; 31: 372–376.

72. Varela AI, Ritchie PA, Smith PJ. Low levels of global genetic differentiation and population expansion in the deep-sea teleost *Hoplostethus atlanticus* revealed by mitochondrial DNA sequences. Mar Biol. 2012; 159: 1049–1060.

73. Hoarau G, Boon E, Jongma DN, Ferber S, Palsson J, Van der Veer HW, et al. Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). Proc R Soc London Ser B. 2005; 272: 497–503.

74. Pinsky ML, Palumbi SR. Meta-analysis reveals lower genetic diversity in overfished populations. Mol Ecol. 2014; 23: 29–39. doi: 10.1111/mec.12509 PMID: 24372754

75. de Oliveira JN, Gomes G, Rêgo PS, Moreira S, Sampaio I, Schneider H, et al. Molecular Data Indicate the Presence of a Novel Species of *Centropomus* (Centropomidae—Perciformes) in the Western Atlantic. Mol Phylogenet Evol. 2014; 77: 275–280. doi: 10.1016/j.ympev.2014.04.019 PMID: 24792089

76. Phillips NM, Chaplin JA, Morgan DL, Peverell SC. Population genetic structure and genetic diversity of three critically endangered *Pristis* sawfishes in Australian waters. Mar Biol. 2011; 158: 903–915.

77. Bellwood DR, Wainwright PC. The History and Biogeography of Fishes on Coral Reefs. In: Sale PF, editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. New York, USA: Academic Press; 2002. pp. 5–32.

78. Hewitt GM. Genetic consequences of climatic oscillations in the Quaternary. Philos Trans R Soc Lond B Biol Sci. 2004; 359: 183–195. PMID: 15101575

79. Hoareau TB, Boissin E, Berrebi P. Evolutionary history of a widespread Indo-Pacific goby: the role of Pleistocene sea-level changes on demographic contraction/expansion dynamics. Mol Phylogenet Evol. 2012; 62: 566–572. doi: 10.1016/j.ympev.2011.10.004 PMID: 22037473

80. Eytan RI, Hellberg ME. Nuclear and mitochondrial sequence data reveal and conceal different demographic histories and population genetic processes in Caribbean reef fishes. Evolution 2010; 64: 3380–3397. doi: 10.1111/j.1558-5646.2010.01071.x PMID: 20584072

81. Rocha LA, Rocha CR, Robertson DR, Bowen BW. Comparative phylogeography of Atlantic reef fishes indicates both origin and accumulation of diversity in the Caribbean. BMC Evol Biol. 2008; 8: doi: 10.1186/1471-2148-8-157.

82. Sachs JP, Anderson RF, Lehman SJ. Glacial surface temperatures of the southeast Atlantic Ocean. Science. 2001; 293: 2077–2079. PMID: 11557890

83. Wallace RK. Thermal acclimation, upper temperature tolerance, and preferred temperature of juvenile yellowtail snappers, *Ocyurus chrysurus* (Bloch)(Pisces: Lutjanidae). Bull Mar Sci. 1977; 27: 292–298.

84. Fernández Iriarte PJ, Pía Alonso M, Sabadin DE, Arauz PA, Iudica CM. Phylogeography of weakfish *Cynoscion guatucupa* (Perciformes: Sciaenidae) from the southwestern Atlantic. Sci Mar. 2011; 75: 701–706.

85. Barreto AMF, Bezerra FHR, Suguio K, Tatumi SH, Yee M, Paiva RP, et al. Late Pleistocene marine terrace deposits in northeastern Brazil: sea-level change and tectonic implications. Palaeogeogr Palaeoclimatol Palaeoecol. 2002; 179: 57–69.

86. Khodri M, Leclainche Y, Ramstein G, Braconnot P, Marti O, Cortijo E. Simulating the amplification of orbital forcing by ocean feedbacks in the last glaciation. Nature. 2001; 410: 570–574. PMID: 11279492

87. Selkoe KA, Gaggiotti OE, TOBO Laboratory, Bowen BW, Toonen R. Emergent patterns of population genetic structure for a coral reef community. Mol Ecol. 2014; 23: 3064–3079. doi: 10.1111/mec.12804 PMID: 24866831

88. Gaither MR, Toonen RJ, Bowen BW. Coming out of the starting blocks: extended lag time rearranges genetic diversity in introduced marine fishes of Hawai'i. Proc Biol Sci. 2012; 279: 3948–3957. doi: 10.1098/rspb.2012.1481 PMID: 22874747

89. Freitas MO, de Moura RL, Francini-Filho RB, Minte-Vera CV. Spawning patterns of commercially important reef fish (Lutjanidae and Serranidae) in the tropical western South Atlantic. Sci Mar. 2011; 75: 135–146.