GENETIC STRUCTURE AND DEMOGRAPHIC HISTORY OF STRIPED WEAKFISH *CYNOSCION GUATUCUPA* (SCIAENIDAE) FROM THE SOUTHWESTERN ATLANTIC

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Running head: GENETIC STRUCTURE OF *CYNOSCION GUATUCUPA*

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ABSTRACT

In South America, the Pleistocene was characterized by important environmental changes related to glacial cycles, which had significant effects on the evolutionary history of several marine species. To evaluate the pattern of demographic history and the genetic structure of striped weakfish *Cynoscion guatucupa*, a 401 bp fragment of the mitochondrial Cytochrome b gene was sequenced from 92 individuals from three coastal areas in Argentina and one in Brazil in the southwestern Atlantic. Haplotype diversity was high, while nucleotide diversity was low among all sampling sites. The star-like pattern was not phylogeographically structured, in agreement with the AMOVA analysis. The Fu's test was negative and highly significant while the mismatch analysis yielded a unimodal distribution indicating population expansion. The mutation rate of Cytochrome *b*, calibrated with pairs of species of *Cynoscion* found on both sides of the Isthmus of Panama was estimated at 0.006 substitutions per million years. The Bayesian skyline plot was used to date changes in population size through time and revealed a coalescence time of 155,000 years. *Cynoscion guatucupa* exhibited a mutation accumulation pattern associated with a rapid population growth after a period of low effective population size, probably linked to climatic changes in the late Pleistocene.

Key-words: genetic structure, cytochrome b, marine fish, Pleistocene, mutation rate.

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RESUMEN

Durante el Pleistoceno se registraron en Sudamérica importantes cambios climáticos relacionados con ciclos de glaciaciones que probablemente han tenido efectos significativos en la historia evolutiva de muchas especies marinas. Para evaluar el patrón de demografía histórica y la estructura genético poblacional de la pescadilla de red (*Cynoscion guatucupa*) se analizó una secuencia de 401 pb del Citocromo b del ADN mitocondrial de 92 individuos de tres áreas costeras en Argentina y una de Brasil en el Atlántico suroccidental. La diversidad haplotípica fue alta y la diversidad nucleotídica fue baja para todos los sitios de muestreo. La topología en forma de estrella en base a los haplotipos mitocondriales muestra un patrón no estructurado geográficamente en concordancia con el análisis de AMOVA. El test de Fu fue negativo y altamente significativo, mientras que el análisis de "mismatch distribution" produjo una distribución unimodal, indicando expansión poblacional. La tasa de mutación del Citocromo b, calibrada a partir de la comparación de la divergencia entre especies del género *Cynoscion* encontrados en ambos lados del Istmo de Panamá fue estimada en 0.006 sustituciones por millón de años. Para datar el cambio del tamaño poblacional a través del tiempo se usó "Bayesian Skyline Plot" estimando un tiempo a la coalescencia de 155,000 años. *Cynoscion guatucupa* mostró un patrón de acumulación de mutaciones asociado a un rápido crecimiento poblacional luego de un cuello de botella, probablemente relacionado con un evento de cambio climático ocurrido en el Pleistoceno medio-tardío.

Palabras Clave

estructura genética, citocromo b, peces marinos, Pleistoceno, tasa de mutación

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INTRODUCTION

Marine fish characterized by high dispersal usually display a weak phylogeographic structure (Avise 1987); isolation by distance only occurs at large geographical scales (Matschiner et al 2010). This pattern is associated with the general absence of dispersive barriers as well as with the high level of spatial connectivity among environments (Grant & Bowen 1998). However, the climatic changes associated with glaciations produced variations in sea temperature, current changes and/or loss of coastal habitats, which played a key role in the evolutionary history of marine species (Hewitt 2000; Rabassa et al. 2011). During the Pleistocene, 10,000 years - 2 million years ago, global glaciation cycles affected the genetic structure of terrestrial as well as of marine species (Hewitt 2000). In South America, three major glaciations occurred in the last 250,000 years (Rabassa et al. 2005, 2011). These climatic changes in the southwestern Atlantic affected the distribution patterns and abundance of marine fishes. Earlier studies using mitochondrial DNA sequences (control region and Cytochrome b) in South American coastal fishes account for phylogeographic patterns that are not geographically structured in *Cynoscion acoupa* (Rodrigues et al. 2008), Macrodon *ancylodon* (Santos et al. 2006), *Pagrus pagrus* (Porrini et al. 2015) and *Eleginops maclovinus* (Ceballos et al. 2012) or with a moderate or low genetic structuring in *Odontesthes argentinensis* (Beheregaray & Sunnucks, 2001), *Brevoortia aurea* (García et al., 2008), *Micropogonias furnieri* (Pereira et al., 2009), and *Paralichthys orbignyanus* (Fernández Iriarte et al., 2014). Likewise, there is clear evidence of population expansion, in several cases, related to the climatic and geographic changes that took place in marine and coastal regions during the Pleistocene.

Striped weakfish *Cynoscion guatucupa* Cuvier, 1830 is a widely spread demersal fish predominantly found on southwestern Atlantic coasts, ranging from Rio de Janeiro, Brazil

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(22° S), to Chubut province, Argentina (43° S). It inhabits coastal areas and part of the continental shelf, and is found in sea and estuarine waters, being typically caught in Brazil, Uruguay and Argentina (Cousseau & Perrotta 2004). Argentine landings are from catches made from two main fishing areas: the Argentine-Uruguayan Common Fishing Zone (34° S to 39° S), and the southern area of Buenos Aires province (El Rincón) (39° S to 41° S), being the second most important species (Ruarte et al. 2004). Earlier studies focusing on Argentine samples of *C. guatucupa* and using the mitochondrial Control Region postulate that there is no pattern of genetic structure among Argentinean populations, though an historical population expansion would have occurred (Sabadin et al. 2009; Fernández Iriarte et al. 2011).

The objectives of this study were: (i) to analyze *C. guatucupa* genetic structure using Cytochrome b marker across its distribution in the southwestern Atlantic, including a sample from Brazil (reaching most of the species distribution), (ii) to calibrate the mutation rate for this mitochondrial gene fragment using this data set, and iii) to infer the main historical demographic events in the species.

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MATERIALS AND METHODS

Sampling, DNA extraction, PCR amplification and sequencing

Samples of muscle tissue kept in ethanol were obtained from adult individuals caught in El Rincón (39° S; 61° W) (n = 25), Mar Chiquita (37° S; 57º W) (n = 21), and Samborombón (36° S; 56° W) (n = 28) on Buenos Aires province coast (Argentina) and in Ubatuba (23º S; 45º W) (n = 18) on the Brazilian coast (Figure 1). Specimens were obtained from local

fishermen and/or from landing samplings collected from different ports. DNA was extracted using Chelex 100 method (Estoup et al. 1996). A small muscle piece of each individual (100-150 mg) was incubated with 500 µl of Chelex 100 at 10 % solution and 25 µl of Proteinase K (20 µg/µl) for 1 hour at 56 ºC followed by 100 ºC for 15 min. The amplification of a partial fragment of Cytochrome b (Cyt b) was conducted using primers Glu-L-CP and CB2-H (Aboim *et al*., 2005). PCR conditions using 50 µl reaction volumes were: 5 µl of 10× buffer, 3.6 µl of Cl2Mg (25 mM), 5 µl of each primer (200 mM), 6 µl of dNTPs (100 mM), 1 µl of Taq polymerase (5 U/µl), and 5 µl of genomic DNA. The reaction volume was completed with water. PCR was performed in a GeneAmp PCR system 2700 thermocycler (Applied Biosystems) at 94 °C for 4 min, followed by 30 cycles at 94 °C for 50 s, 50 °C for 30 s and 72 °C for 1 min, with a final extension of 72 °C for 5 min. The PCR product was purified and sequenced in MACROGEN Korea (http://www.macrogen.com/).

Population genetic analysis

Sequences were manually aligned using PROSEQ (Filatov 2002). For each sampling site, haplotype (h) and nucleotide (π) diversity were estimated using DNAsp v5 (Librado & Rozas 2009). Likewise, to detect historical demographic changes, the D (Tajima 1989) and F_s (Fu 1997) neutrality tests were calculated to discriminate mutation/drift equilibrium and to evaluate the hypothesis of population expansion through the significant excess of low-frequency haplotypes. These parameters were calculated using ARLEQUIN 3.11 (Excoffier et al. 2005). Mismatch distribution was also estimated with DNAsp, and it was compared with the distribution expected under a model of sudden population expansion. Deviations

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150 from the model were evaluated by calculating the R_2 index (Ramos-Onsins and Rozas,
151 2002). Those populations that have undergone large expansion are expected to exhibit
152 unimodal mismatch distributions with a low R_2 value; while stable populations produce a
153 variety of multimodal distributions with a high R_2 index. The significance of D, F_s and R_2
154 indices was evaluated on 10,000 simulations. The genetic structure of *C. guatucupa* was
155 assessed by the Analysis of Molecular Variance (AMOVA), and pairwise genetic distances
156 were estimated to assess fixation indices (Φ_{ST}) among all population pairs (10,000
157 permutation) in ARLEQUIN. The haplotype median-joining network was constructed using
158 Network 4.6 (Bandelt et al. 1999).
159
160 *Molecular clock and coalescence time*
161 The pair of *Cynoscion* species found on both sides of the Isthmus of Panama has evolved in
162 isolation for about 3.5 million years (Coates et al. 1992), and could be used to calibrate the
163 molecular clock (Bermingham & Lessios 1993). Hence, by comparing the variations within
164 and among *Cynoscion* genus species for Cyt b sequence (Vergara-Chen et al. 2009), less
165 biased estimates of the mutation rate could be calculated, and historical demographic
166 changes occurring in the glaciations could be inferred with greater precision. Cyt b
167 sequences were obtained from GenBank for *C. reticulatus* Günther, 1864 (GQ220005.1),
168 *C. nothus* Holbrook, 1848 (GQ220006.1), *C. phoxocephalus* Jordan and Gilbert, 1882
169 (GQ220010.1) and *C. leiarchus* Cuvier, 1830 (GQ219999.1). The molecular clock was
170 calculated from the sequences of *Cynoscion* genus using Kimura 2-parameter distance
171 (Kimura 1980).

Expansion time was directly estimated from mismatch distribution with the statistic τ (tau) and translated into absolute time in years (t), using the equation t = τ/2μk, where μ is the mutation rate per year and k is the number of nucleotides of the sequence analyzed. Confidence intervals for t estimates were obtained using a parametric bootstrap approach in ARLEQUIN. On the other hand, the substitution model of Cyt b in *C. guatucupa* was determined using jModelTest 0.1.1 (Posada 2008) and, based on such model, expansion time was calculated using the Bayesian Skyline Plot (BSP) in BEAST (Drummond & Rambaut 2007). BSP analysis was performed using a relaxed molecular clock, with three runs of 10 million steps (MCMC) each, where trees and parameters were sampled every 1,000 steps. The fact that the ESS values estimated with Tracer were > 200 (Drummond et al. 2012) allowed determining that the two independent runs converged on the same distribution in the MCMC run. Only one of the three runs was plotted. To estimate coalescence time, the calibrated mutation rate was applied.

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RESULTS

Molecular diversity, demography and network

A 401 bp fragment of the mitochondrial Cyt b gene was sequenced for 92 individuals. Thirty one different haplotypes (GenBank Accession numbers KR086363-KR086393), 27 substitutions (22 transitions and 5 transversions) were observed. The average number of nucleotide differences between pairs of sequences (*k*) was 1.504 (Table 1). The molecular diversity patterns for all individuals yielded a haplotype diversity (*h*) (mean ± SD) = 0.822 ± 0.034 and a nucleotide diversity (π) (mean ± SD) = 0.004 ± 0.003 (Table 1). Haplotype

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diversities decreased from South to North ranging from 0.903 ± 0.001 in RIN to 0.569 ± 0.018 in UBA, respectively.

The statistical test of neutrality (D) was negative and significant for each site except for Samborombón when all samples were analyzed together; the Fu's *F* was negative and statistically significant in all sites and at a total level (Table 1). The global mismatch analysis yielded a unimodal distribution curve (Figure 2) associated with the low R_2 value (R_2 = 0.026, P <0.05).

The network of Cyt b haplotypes displayed no evidence of a phylogeographic structure and showed a star-like pattern with two highly represented central haplotypes, differentiated from each other by a single mutation step (Figure 3). AMOVA indicated that the largest genetic variation was within groups and not among them (ϕ_{ST} = -0.011, df = 3, 88, P = 0.81). Additionally, no significant ϕ_{ST} values (P > 0.05) were noticed among site pairs: UBA-SAM (ϕ_{ST} = -0.013), UBA-MCH (ϕ_{ST} = -0.010); UBA-RIN (ϕ_{ST} = -0.007), SAM-MCH (ϕ_{ST} = -0.010), SAM-RIN (ϕ_{ST} = -0.010) and MCH-RIN (ϕ_{ST} = -0.014).

Mutation rate, Tau and BSP

Regarding *C. reticulatus* - *C. nothus* sequences, the genetic distance was of 0.052 and for *C. phoxocephalus* - *C. leiarchus*, it was of 0.034, thereby yielding a mean genetic distance of 0.043 between species. A mutation rate of 1.2 % between species of the *Cynoscion* genus was estimated on the basis of such distance. Given the fact that the mutation rate within lineages is half the rate between lineages (Bowen et al. 2006), a mutation rate of 0.6 % per million years was established.

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The τ (*tau*) parameter, in turn, was 1.55 (confidence interval, α = 0.050: 1.15-1.99), estimating the expansion time at 320,000 (230,000- 410,000) years ago.

The substitution rate of Cyt b using Akaike information criterion was adjusted to the Hasegawa, Kishino and Yano model (HKY) more the proportion of invariable sites (I). BSP, using a mutation rate of 0.006 substitutions per million years, revealed a significant reduction in population size followed by an expansion about 155,000 years ago (Figure 4).

Moreover, for BSP construction, confidence intervals for estimates of this marker in several fish species (1- 1.8 %) (Pfeiler et al. 2008) were used. These substitution rates of 0.009 substitutions per million years (maximum) and 0.005 substitutions per million years (minimum), yielded coalescence values ranging from 105,000 to 185,000 years, respectively (Figure 4).

Discussion

The absence of a genetic structure across *C. guatucupa* distribution in the southwestern Atlantic may be explained by the fact that this species is characterized by high dispersal and weak barriers to gene flow. Population expansion, estimated by means of the substitution rate of Cyt b, showed demographic processes of low population size after one of the largest Pleistocene glaciations. AMOVA indicated absence of genetic differentiation among sampling sites but most genetic variation among the Cyt b haplotypes was distributed within sites. These results are consistent with those accounted for by Sabadin et al. (2009), which found no differences in two microsatellite loci in individuals from Samborombón and El Rincón. The same result was achieved in the study conducted for the mtDNA control region (Fernández Iriarte et al. 2011), which reported lack of genetic

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structuring among Samborombón, El Rincón and Mar Chiquita. The absence of the genetic differentiation is evident in the haplotype network, in which no genetic structure is observed and individuals from all sites share common haplotypes. This pattern is common in many marine fish species given their life history, characterized by high dispersal and absence of natural barriers (Zane et al. 2006; Pfeiler et al. 2008). When evaluating the individuals from the different sampling sites, no qualitative differences were observed in the molecular diversity values analyzed (h and π) taking, for both cases, a high *h* (> 0.5) and a low π (< 0.005) as a cut-off point (Grant & Bowen 1998). *Pi* (π) values, both at a global and at each sampling site level, were similar and low, while h values were high but showed a pattern of decreasing variability from South (RIN) to North (UBA). The sample from Ubatuba represents the most northern distribution of the species with temperatures that could represent a less favorable habitat. The high haplotype and low nucleotide diversities of *Cynoscion guatucupa* characterize species that have undergone a historical bottleneck, followed by population expansion with accumulation of mutations (Type 2; Grant & Bowen 1998). This pattern was observed in species not genetically structured as *C. guatucupa* (Fernández Iriarte et al. 2011), *Eleginops maclovinus* (Ceballos et al 2012) and *Pagrus pagrus* (Porrini et al. 2015). On the contrary, it is considered that those species with low haplotype and nucleotide diversity have undergone a recent bottleneck or founder effect (Type 1, Grant & Bowen, 1998). This pattern was observed in species such as *M. furnieri* (Pereira et al. 2009) and *P. orbignyanus* (Fernández Iriarte et al. 2014).

The molecular diversity values are also consistent with the unimodal distribution curve and, together with the low R_2 value and the negative Fs values, could indicate that the species underwent demographic expansion. The starting period of population expansion differs depending on the method. The value calculated from τ was of 320,000 years (240,000-

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289 410,000) and from BSP, of 155,000. Despite the fact that it has been suggested that τ tends
290 to underestimate coalescence time (Schneider & Excoffier 1999), said parameter is very
291 sensitive to mismatch curve variations, and small variations could have a great effect on
292 expansion time estimates (Schneider & Excoffier 1999). Another point in this regard is the
293 heterogeneity of the substitution rate along the analyzed sequence and its relevance when
294 expansion time is calculated (Schneider & Excoffier 1999). In view of the fact that BSP
295 uses the substitution model under which the sequence mutates to calculate population
296 expansion, the value obtained would be more representative. The data obtained from BSP
297 for Cyt b (155,000 years) is in agreement with that estimated for the mtDNA control region
298 (190,000 years; Fernández Iriarte et al. 2011). In this sense, changes in population size
299 estimated with Cyt b associate better with the Quaternary climatic changes with a
300 population expansion time that would correspond to the end of the most extensive
301 glaciation event recorded in the last 250,000 years (140,000-180,000 years ago) (Ruzzante
302 et al. 2008). Recent studies using Cyt b in the Patagonian blennie fish *Eleginops*
303 *maclovinus* Cuvier & Valenciennes, 1830 from the southwestern Atlantic estimate a
304 coalescence of 125,000 years (Ceballos et al. 2012), also associated with the end of such
305 cold period.

306 The substitution rate of Cyt b in fishes was five to ten times lower than that of the
307 control region (Bargelloni et al. 2003), while in *C. guatucupa* this value was 8.3 times
308 smaller. In general, the mean mutation rate of the estimated control region was 0.05
309 substitutions per million years (Bowen et al. 2006; Ruzzante et al. 2008; Fernández Iriarte
310 et al. 2011). In this sense, the mutation rate reported here, calibrated by the divergence
311 between species, increases the reliability of the estimated times, and is consistent with that
312 used for other fish groups, being very similar to that applied to Cyt b in *E. maclovinus*

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(0.0056), in line with the fossil record. Even though the real starting period of population expansion may be slightly different, present data suggests that the glacial period could have been particularly relevant for the distribution and abundance of marine species in the southwestern Atlantic (~~Zane et al. 2006; Matschiner et al. 2010~~).

Comentario [CU1]: No son citas pertinentes aquí, ya que ninguno de los dos trabajos habla de la influencia de las **glaciaciones sobre los peces del Atlántico sur.**

Comentario [DA2]: Estoy de acuerdo entonces saco la cita de Matschiner de la bibliografia

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