

## FULL LENGTH RESEARCH PAPER

**Mitochondrial DNA reveals population structuring in *Macrodon atricauda* (Perciformes: Sciaenidae): a study covering the whole geographic distribution of the species in the southwestern Atlantic**Rosa Rodrigues<sup>1</sup>, Simoni Santos<sup>2</sup>, Manuel Haimovici<sup>3</sup>, Ulrich Saint-Paul<sup>4</sup>, Iracilda Sampaio<sup>1</sup>, and Horacio Schneider<sup>1</sup>

<sup>1</sup>Laboratório de Genética e Biologia Molecular, Instituto de Estudos Costeiros, Universidade Federal do Pará, Campus de Bragança, Pará, Brasil, <sup>2</sup>Laboratório de Microbiologia do Pescado, Instituto de Estudos Costeiros, Universidade Federal do Pará, Pará, Brasil, <sup>3</sup>Laboratório de Recursos Pesqueiros Demersais e Cefalópodes, Instituto de Oceanografia, Universidade Federal do Rio Grande, Rio Grande, RS, Brasil, and <sup>4</sup>Leibniz Zentrum für Marine Tropenökologie (ZMT), Bremen, Germany

**Abstract**

We investigated the genetic structure and diversity of *M. atricauda*, based on 266 specimens collected off the coast of southern Brazil and Argentina at seven locations, covering the whole geographic distribution of this species. A DNA sequence alignment of 904 base pairs of the mitochondrial Control Region revealed a total of 85 haplotypes.  $F_{ST}$  analyses suggest that *M. atricauda* does not comprise a single demographic stock. Two different genetic units are identified, which possibly are related to ecological adaptations of the species within its range. Genetic diversity, Bayesian analysis of population structure, and significant negative results for the D and FS tests indicate that *M. atricauda* populations have undergone recent expansion. The spatial distribution of genetic variation seems to be related to historical colonization from south to north, followed by expansion.

**Keywords**

Control region, genetic structure, *Macrodon atricauda*, southern king weakfish, southwestern Atlantic

**History**

Received 14 January 2013  
Revised 28 March 2013  
Accepted 31 March 2013  
Published online 7 May 2013

**Introduction**

The southern king weakfish, *Macrodon atricauda* Günther, 1880, is a sciaenid fish that was revalidated (Carvalho-Filho et al., 2010). Previously, this species was synonymized with *Macrodon ancylodon* Bloch and Schneider, 1801, which is distributed between Venezuela and Argentina (Cousseau & Perrota, 1998; Menezes et al., 2003). However, in an analysis based on genetic (Santos et al., 2003, 2006; Sampaio et al., 2010), meristic, and morphological data (Carvalho-Filho et al., 2010), it was concluded that this geographic area contains two species, with *M. ancylodon* distributed between Venezuela and the eastern Brazilian state of Bahia and *M. atricauda* distributed from the southeastern Brazilian state of Espírito Santo southwards to Argentina.

The southern king weakfish is a demersal species that inhabits coastal and estuarine waters. The fishery for *Macrodon atricauda* is economically important throughout the species geographic range, especially in southern Brazil (Haimovici, 1998) and to a lesser extent in Uruguay and Argentina (Cousseau & Perrota, 1998). Migration occurs over relatively short distances and spawning occurs primarily in the mouths of rivers, as larvae and juveniles are dependent on estuarine ecosystems for their development (Juras & Yamaguti, 1989; Yamaguti, 1979). Much of the biological and ecology of the species has been studied in

detail, including its feeding behavior (Juras & Yamaguti, 1985), growth and longevity (Cardoso & Haimovici, 2011; Yamaguti & Santos, 1966), mortality (Yamaguti, 1968), and reproductive patterns (Juras & Yamaguti, 1989; Militelli & Macchi, 2004). However, population genetic data are limited to studies of Santos et al. (2003) and Santos et al. (2006) and Sampaio et al. (2010).

Using meristic and morphometric variables, Yamaguti (1979) distinguished four stocks of *M. atricauda* from southeastern and southern Brazil: Espírito Santo (1), Rio de Janeiro (2), São Paulo/Paraná/Santa Catarina (3), and Rio Grande do Sul (4). Analyzing DNA sequences of the mitochondrial cytochrome b gene in *M. atricauda* from the whole of the range of the species, between Espírito Santo and Argentina, Santos et al. (2006) recorded significant differences among three demographic stocks, represented by Espírito Santo (1), Paraná/São Paulo (2), and Santa Catarina/Rio Grande do Sul/Argentina (3). In the present study, a more variable mitochondrial marker, the Control Region or D-Loop, was analyzed to better clarify sub-population genetic structure and diversity throughout across the geographic range of *M. atricauda* aiming to validate the four groups recovered by Yamaguti (1979) or the ones revealed by the genetic analyses of Santos et al. (2006).

**Material and method****Sampling**

Two hundred and sixty-six *M. atricauda* specimens were collected from southern and southeastern Brazil, including the states of Espírito Santo (Vitória,  $N=5$ ), Rio de Janeiro (Niterói,  $N=7$ ), São Paulo (Santos,  $N=65$ ), Paraná (Paranaguá,  $N=35$ ), Santa Catarina (Itajaí,  $N=56$ ), Rio Grande do Sul (Rio Grande,  $N=69$ ), as well as Argentina ( $N=29$ ; Figure 1).



Figure 1. Collection locations for *Macrodon atricauda* of the southwestern Atlantic. ES = Espírito Santo, RJ = Rio de Janeiro, SP = São Paulo, PR = Paraná, SC = Santa Catarina, RS = Rio Grande do Sul and ARG = Argentina. Rectangle represents the Brazil Malvinas Confluence zone. Groups 1, 2, 3 and 4 of Yamaguti (1979) are identified.

### Molecular techniques

Total genomic DNA was extracted from muscle tissue, using proteinase K and phenol/chloroform protocol (Sambrook & Russell, 2001). A segment of 904 base pairs of the mitochondrial Control Region was amplified using the primers L1 5'-CCTAACTCCCAAAGCTAGGTATTC-3' and H 5' TTCTTCCCY(CT)W(AT)CTCATW(AT)TGCTACAC-3'. The PCR was run in a total volume of 25  $\mu$ l, with each reaction containing 1–2  $\mu$ l of total DNA (50–100 ng/ $\mu$ l), 4  $\mu$ l of dNTP (1.25 mM), 2.5  $\mu$ l of buffer (10x), 1  $\mu$ l

of  $MgCl_2$  (50 mM), 0.2  $\mu$ l each primer (200 ng/ $\mu$ l), 0.2  $\mu$ l of Taq DNA polymerase, 5U/ $\mu$ l (Invitrogen, EUA), and purified water to complete the final volume. The amplification protocol was an initial cycle of 3 min at 94  $^{\circ}C$ , followed by 35 cycles of 94  $^{\circ}C$  for 30 seconds, 59.5  $^{\circ}C$  for 1 min, and 72  $^{\circ}C$  for 2 min, followed by a final extension of 7 min at 72  $^{\circ}C$ . Sequencing reactions were conducted using a Big Dye v3.1 terminator kit and products separated and visualized on an ABI 3500 automatic sequencer (Applied Biosystems, Foster City, CA, USA). All haplotype sequences are available on GenBank (Access Numbers xxxxxx-xxxxxx).

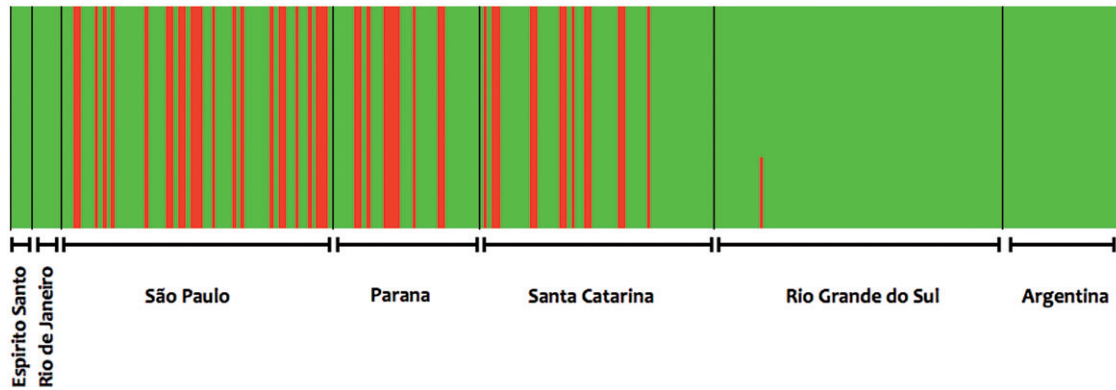


Figure 2. Results of genetic assignment of individuals analysis based on Bayesian method implemented in the programs *BAPS* under mixture of group of populations.

Table 1. Genetic diversity and neutrality test for the 904 bp of the control region from *Macrodon atricauda*.

Locality	N	NH	Haplotype diversity ( <i>h</i> )	Nucleotide diversity ( $\pi$ )	Tajima D test	Fu's <i>F<sub>s</sub></i> test
Espírito Santo	5	4	0.900 ± 0.161	0.0017 ± 0.0014	-1.093	-1.404
Rio de Janeiro	7	6	0.952 ± 0.095	0.0034 ± 0.0023	-0.862	-2.128
São Paulo	65	33	0.907 ± 0.024	0.0035 ± 0.0020	-2.031*	-26.227*
Paraná	35	16	0.826 ± 0.058	0.0027 ± 0.0017	-1.603*	-8.603*
Santa Catarina	56	29	0.857 ± 0.045	0.0032 ± 0.0019	-1.939*	-24.882*
Rio Grande do Sul	69	18	0.591 ± 0.070	0.0008 ± 0.0007	-2.397*	-21.513*
Argentina	29	10	0.576 ± 0.109	0.0011 ± 0.0008	-2.290*	-6.947*
All	266	85	0.798 ± 0.025	0.0025 ± 0.0015	-2.504*	-26.697*

\* $p < 0.01$ ; N, number of individuals; NH, number of haplotypes. Haplotype gene diversity *h* and Nucleotide diversity per site  $\pi$  include standard deviation of each estimate.

### Population genetic analyses

The control region sequences were edited and aligned using the BIOEDIT 7.0.9 software (Hall, 1999). Haplotype and nucleotide diversity (Nei, 1987) of *M. atricauda* were calculated in DNASP 5.10 (Librado & Rozas, 2009) and ARLEQUIN 3.5 (Excoffier & Lischer, 2010).

The number of base substitutions per site from averaging over all sequence pairs between groups was estimated using the Kimura 2-parameter model. The analysis involved 266 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 904 positions in the final data set. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

The program BAPS6 (Corander et al., 2003; Corander & Martinen, 2006) was used to detect clusters of genetically similar populations. When testing for population clusters, 10 replicates for every level of *k* (*k* is the maximum number of clusters) up to *k* = 10 were run. The number of reference individuals was set to 200 and admixture analysis were run 50 times per individual.

Differentiation between populations was tested using pairwise fixation index ( $F_{ST}$ ) and AMOVA was used to look at differentiation across all populations, these estimated using ARLEQUIN 3.5 (Excoffier et al., 1992); significance was assessed based on 10,000 random permutations. Isolation by distance (IBD), the correspondence between genetic ( $F_{ST}$ ). Inferences on the demographic history of *M. atricauda* were based on Fu's  $F_S$  (Fu, 1997) and Tajima's *D* (Tajima, 1989) tests, which were run in ARLEQUIN 3.5 with 10,000 simulations.

### Results and discussion

A total of 904 bps of the mitochondrial control region (mtDNA CR) was sequenced in 266 specimens of *M. atricauda* collected from seven locations. The alignment disclosed 84 polymorphic

Table 2. Matrix of pairwise  $F_{ST}$  values (below the diagonal) for population differentiation in *Macrodon atricauda* and estimates of evolutionary divergence over sequence pairs between groups.

Pairwise $F_{ST}$	Evolutionary divergence				
	São Paulo	Paraná	Santa Catarina	Rio Grande do Sul	Argentina
São Paulo	–	0.4500	0.1145	0.000*	0.00010*
Paraná	-0.0031	–	0.5840	0.000*	0.00307*
Santa Catarina	0.0088	-0.0068	–	0.000*	0.00614*
Rio Grande do Sul	0.1710*	0.1479*	0.0918*	–	0.36996
Argentina	0.1211*	0.0938*	0.0551*	0.0019	–
Divergences (K2P)					
São Paulo	<b>0.00338</b>				
Paraná	0.00295	<b>0.00254</b>			
Santa Catarina	0.00321	0.00205	<b>0.0029</b>		
Rio Grande do Sul	0.00252	0.00190	0.0025	<b>0.00076</b>	
Argentina	0.00255	0.00194	0.00210	0.00084	<b>0.00091</b>

\* $p < 0.01$  = Significance of  $F_{ST}$  values (above the diagonal): Numbers in boldface are intra population divergences.

sites (52 transitions, 28 transversions, and 5 indels), retrieving 85 haplotypes.

The most common haplotype, H1 occurred in 117 of the 266 individuals (43.98%) and all 7 populations (Figure 2). Haplotype 11 (H11) was second in abundance but was observed only in specimens from São Paulo, Paraná, and Santa Catarina. Connection of H1 by one or two mutational steps to a large number of low-frequency or rare haplotypes (Figure 2), is consistent with the coalescence hypothesis of Posada & Crandall (2001) that haplotypes with a higher frequency and wider geographic distribution tend to have a larger number of mutational connections.

Nucleotide diversity was low in all samples, ranging from 0.0008 (Rio Grande do Sul) to 0.0035 (São Paulo); haplotype

Table 3. Frequency of the 85 haplotypes of *Macrodon atricauda* sampled in seven localities of South American Coast.

Haplotype	Espírito Santo	Rio de Janeiro	São Paulo	Paraná	Santa Catarina	Rio Grande do Sul	Argentina	Total
1	2	2	15	14	21	44	19	117
2	1		1		1			3
3	1							1
4	1					1		2
5		1						1
6		1				3	2	6
7		1						1
8		1						1
9		1						1
10			1					1
11			13	5	3			21
12			1					1
13			1					1
14			1					1
15			1					1
16			1	1	1			3
17			1		1			2
18			4		2			6
19			2	1	1			4
20			2	1				3
21				1				1
22			1			2		3
23			1			4	1	6
24			2					2
25			1	1	1			3
26			1					1
27			1					1
28			1					1
29			1					1
30			1					1
31			1					1
32			1					1
33			1					1
34			1					1
35			1		1			2
36			1					1
37			1					1
38			1					1
39			1					1
40			1				1	2
41				1				1
42				1				1
43				1				1
44				2	3			5
45				1				1
46				1				1
47				1				1
48				1				1
49				2	1			3
50				1				1
51					1			1
52					1			1
53					1			1
54					1			1
55					1			1
56					2			2
57					1			1
58					1			1
59					1			1
60					2			1
61					1			1
62					1		1	2
63					1			1
64					1	1		2
65					1			1
66					1			1
67					1			1
68					1			1
69						1		1
70						1		1

(continued)



Table 3. Continued

Haplotype	Espírito Santo	Rio de Janeiro	São Paulo	Paraná	Santa Catarina	Rio Grande do Sul	Argentina	Total
72						1		1
72						1		1
73						1		1
74						1		1
75						1		1
76						1		1
77						1		1
78						3		3
79						1		1
80						1		1
81							1	1
82							1	1
83							1	1
84							1	1
85							1	1
Total	5	7	64	36	56	69	29	266

diversity, however, was moderate to high (Table 1), ranging from  $h = 0.576$  (Argentina) to  $h = 0.952$  (Rio de Janeiro). This pattern of haplotype and nucleotide diversity is typical of populations that have undergone recent expansion (Grant & Bowen, 1998). The genetic diversity recorded in the present study for *M. atricauda* was similar to that found for the mtDNA CR in a second commercially valuable sciaenid, *Cynoscion acoupa* ( $h = 0.89$  and  $\pi = 0.003$ ) by Rodrigues et al. (2008) but lower than the values recorded in some other marine fishes (Gomes et al., 2008; Lu et al., 2006; Wang et al., 2008), including a number of sciaenid taxa (Fernández Iriarte et al., 2011; Kim et al., 2010; Seyoum et al., 2000).

Fu's  $F_S$  and Tajima's  $D$  were significantly negative for all samples except for those from Espírito Santo and Rio de Janeiro (Table 1), where values for both tests were negative but non-significant, though sample sizes were small. Overall, these significantly negative values indicate that *M. atricauda* has undergone a demographic expansion (Fu, 1997; Ramírez-Soriano et al., 2008; Tajima, 1989), a conclusion supported by the general pattern of diversity and the star-shaped haplotype network (not showed). Evidence of demographic expansion of *M. atricauda* is consistent with the results of Santos et al. (2006), who confirmed this process based on the mitochondrial cytochrome b gene.

Based on the analysis of the haplotype abundance of the mtDNA CR, the most common haplotype has its higher frequency among the southern locations (Arg and RGS), suggesting recent expansion in the south towards the north. (see Table 1). As the species advanced to northern localities, genetic diversity would have increased. This is consistent with other evidences that climatic fluctuations during the Pleistocene were important factors determining the origin, distribution, and expansion of marine fishes (Fernández Iriarte et al., 2011; Larmuseau et al., 2009; Santos et al., 2006).

The fixation index ( $F_{ST}$ ) ranged from  $-0.0031$  to  $0.1710$  (Table 2), with samples from Espírito Santo and Rio de Janeiro excluded due to small sample size. Significant differences observed between pairs of populations indicate two principal groups, one comprised by São Paulo, Paraná, and Santa Catarina, and other by Rio Grande do Sul and Argentina. The AMOVA indicated that 88.84% of the genetic variance was derived from within-group differences ( $F_{ST} = 0.1115$   $p < 0.0001$ ), whereas 10.88% of the genetic variance was due to differences between the southeastern (São Paulo, Paraná, and Santa Catarina) and the southernmost groups (Rio Grande do Sul and Argentina;  $F_{CT} = 0.1088$   $p = 0.005$ ). These significant  $F_{ST}$  values observed

for the southeastern populations (São Paulo, Paraná, and Santa Catarina) emphasize their inclusion in a distinct group.

All BAPS analyses (clustering on the individual or population level) resulted in two genetic distinct clusters: one constituted by Espírito Santo, São Paulo, Paraná and Santa Catarina; and other including Rio Grande do Sul and Argentina (Figure 2).

Findings from  $F_{ST}$  analysis also indicate the same clusters identified by the Bayesian analysis. These two groups correspond to groups 3 and 4 recovered by Yamaguti (1979) including Argentina in this later one. However, groups named 1 and 2 (Espírito Santo and Rio de Janeiro) in Yamaguti (1979), could not be confirmed by Bayesian analysis in the present article. Similarly, the findings of Santos et al. (2006) based on cytochrome b sequences that recovered the group 1 (group 2 = RJ was not represented) also could not be confirmed. Perhaps the small sample size (SP = 12, PR = 11; SC = 9; RS = 8; Argentina = 11) used for Santos et al. (2006) could be responsible for the differences observed.

*Macrodon* has been confirmed as estuarine dependent fish on surveys of ictioplankton (Barletta-Bergan et al., 2002), and in studies of estuarine fish assemblages in western Atlantic (Barletta et al., 2005; Lorenzo et al., 2011; Passos et al., 2012; Plavan et al., 2010). The existence of distinct stocks, confirmed by both morphometric and meristic analyzes as well as genetic analyses, reinforce the limited migration capacity of *M. atricauda*. Therefore, limited gene flow may be a consequence of this behavior, which seems to be the case. The pioneering studies of the ecology and morphology of the southern king weakfish indicate that this species undertakes limited migrations and spawns during distinct periods and at different sites along the coast of southern Brazil (Santos & Yamaguti, 1965; Yamaguti, 1979).

In addition, it is well known that temperature and salinity of the water have strong influence on population structuring process on marine fishes, and several studies have documented the influence of these factors over sciaenid fish assemblages off the coast of southern Brazil (Araújo et al., 2002, 2006; Muto et al., 2000; Rocha et al., 2010; Schmidt & Dias, 2012). According to Matano et al. 2010, the oceanic circulation over the southwestern Atlantic shelf is influenced by large tidal amplitudes, substantial freshwater discharges, high wind speeds, and most important by its proximity to Brazil and Malvinas Currents. The Malvinas Current (MC) is a branch of the Antarctic Circumpolar Current that flows north along the Argentina coast up to approximately 38°S (Matano et al., 1993). At that latitude the MC collides with the Brazil Current (BC; Piola & Matano, 2001). The collision of

these two currents occurs near the mouth of the La Plata River where it creates a region of extreme variability known as Brazil/Malvinas Confluence (BMC). The Malvinas Current is strong, relatively fresh, and cold, with mean sea surface temperature of 6 °C (Brandini et al., 2000). Thus, when it encounters the weak, warm, southward-flowing Brazil Current at the Brazil-Malvinas confluence, a sharp gradient in temperature and salinity can be observed (Goni et al., 1996). Furthermore, Malvinas waters still penetrate over the Patagonian shelf affecting the regional ecosystems (Matano et al., 2010). The most obvious evidence of this nutrient pumping is the high level of biological activity that is found in the Patagonia region, which is considered a Class I marine ecosystem (Lutz & Carreto, 1991; Matano et al., 2010). Therefore, the sub-tropical shelf front, which is located near 33° S, divides the southwestern Atlantic shelf in two distinct regions: a northern region with warm and salty waters of tropical and subtropical origin, and a southern region with cold and relatively fresh waters of sub Antarctic origin (Piola et al., 2000). The remarkable environmental differences between these two regions may be responsible for the genetic structure observed in *Macrodon atricauda* with the southern form living in the northern region of the Southwestern Atlantic shelf and southernmost form living in the highly variable BMC zone.

## Conclusions

Overall, the results of the present study indicate that the southern king weakfish does not represent a single population throughout its geographic range. Instead, significant genetic differentiation exists between two forms delimited by the BMC zone. The spatial distribution pattern of the genetic diversity of *M. atricauda* strongly indicates an historical process of colonization, with the southernmost population expanding towards the north. These conclusions are important for the development of effective fishery management strategies for *M. atricauda*.

## Acknowledgements

Funding for this research was provided by CNPq (grants 306233/2009-6 to IS and 306233/2009-6 to HS) and FAPESPA (PRONEX 2007 to HS). Rosa Rodrigues was supported by a PhD scholarship from CNPq (141224/2008-8).

## Declaration of interest

The authors report no declaration of interest. The authors alone are responsible for the content and writing of the article.

## References

- Araújo FG, Azevedo MCC, Araújo silva M, Pessanha ALM, Gomes ID, Cruz-filho AG. (2002). Environmental influences on the demersal fish assemblages in the Sepetiba Bay, Brazil. *Estuaries* 25:441–50.
- Araújo FG, Guimarães FJDC, Costa MRD. (2006). Environmental influences on distribution of four Sciaenidae species (Actinopterygii, Perciformes) in a tropical bay at Southeastern Brazil. *Revista Brasileira de Zoologia* 23:497–508.
- Barletta M, Barletta-bergan A, Saint-paul U, Hubold G. (2005). The role of salinity in structuring the fish assemblages in a tropical estuary. *J Fish Biol* 66:45–72.
- Barletta-bergan A, Barletta M, Saint-Paul U. (2002). Structure and seasonal dynamics of larval fish in the Caeté river estuary in North Brazil. *Estuarine, Coastal Shelf Sci* 54:193–206.
- Brandini FP, Boltovskoy D, Piola A, Kocmur S, Röttgers R, Abreu PC, Lopes RM. (2000). Multiannual trends in fronts and distribution of nutrients and chlorophyll in the southwestern Atlantic (30–62°S). Deep-Sea Research, Part I. *Oceanographic Research Papers* 47: 1015–33.
- Cardoso LG, Haimovici M. (2011). Age and changes in growth of the king weakfish *Macrodon atricauda* (Günther, 1880) between 1977 and 2009 in southern Brazil. *Fish Res* 111:177–87.

- Carvalho-filho A, Santos S, Sampaio I. (2010). *Macrodon atricauda* (Günther, 1880) (Perciformes: Sciaenidae), a valid species from southwestern Atlantic, with comments on its conservation. *Zootaxa* 2519:48–58.
- Corander J, Marttinen P. (2006). Bayesian identification of admixture events using multilocus molecular markers. *Mol Ecol* 15:2833–43.
- Corander J, Waldmann P, Sillanpää MJ. (2003). Bayesian analysis of genetic differentiation between populations. *Genetics* 163:367–74.
- Cousseau MB, Perrota RG. (1998). *Peces Marinos de Argentina: biología, distribución y pesca*. Mar del Plata, Argentina, Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP).
- Excoffier L, Lischer HEL. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10:564–7.
- Excoffier L, Smouse PE, Quattro JM. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479–91.
- Fernández Iriarte PJ, Alonso MP, Sabin DE, Arauz PA, Iudica CM. (2011). Phylogeography of weakfish *Cynoscion guatucupa* (Perciformes: Sciaenidae) from the southwestern Atlantic. *Scientia Marina* 75:701–6.
- Fu Y-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915–25.
- Gomes G, Schneider H, Vallinoto M, Santos S, Orti G, Sampaio I. (2008). Can *Lutjanus purpureus* (South red snapper) be “legally” considered a red snapper (*Lutjanus campechanus*)? *Genet Mol Biol* 31:372–6.
- Goni G, Kamholz S, Garzoli S, Olson D. (1996). Dynamics of the Brazil-Malvinas Confluence based on inverted echo sounders and altimetry. *J Geophysical Res* 101:16273–89.
- Grant WAS, Bowen BW. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *J Heredity* 89:415–26.
- Haimovici M. (1998). Present state and perspectives for the southern Brazil shelf demersal fisheries. *Fish Manag Ecol* 5:277–89.
- Hall TA. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Series* 41:95–8.
- Juras AA, Yamaguti N. (1985). Food and feeding habits of king weakfish, *Macrodon ancylodon* (Bloch & Schneider, 1801) caught in the southern coast of Brazil (Lat. 29o to 32o S). *Boletim do Instituto Oceanográfico* 33:149–57.
- Juras AA, Yamaguti N. (1989). Sexual maturity, spawning and fecundity of King weakfish *Macrodon ancylodon*, caught off Rio Grande do Sul State (southern coast of Brazil). *Boletim do Instituto Oceanográfico* 37:51–8.
- Kim JK, Kim YH, Kim M-J, Park JY. (2010). Genetic diversity, relationships and demographic history of the small yellow croaker, *Larimichthys polyactis* (Pisces: Sciaenidae) from Korea and China inferred from mitochondrial control region sequence data. *Animal Cells Syst* 14:45–51.
- Larmuseau MHD, van Houdt KJ, Guelinckx J, Hellemans B, Volckaert FAM. (2009). Distributional and demographic consequences of Pleistocene climate fluctuations for a marine demersal fish in the north-eastern Atlantic. *J Biogeograph* 36:1138–51.
- Librado P, Rozas J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–2.
- Lorenzo MI, Astarloa JMD, Norbis W, Cousseau MB. (2011). Long term fish assemblages as units of management in a temperate estuary (Rio de la Plata - SW Atlantic Ocean). *Brazil J Oceanogra* 59:43.
- Lu CP, Chen CA, Hui CF, Tzeng TD, Yeh SY. (2006). Population genetic structure of the swordfish, *Xiphias gladius* (Linnaeus, 1758), in the Indian Ocean and West Pacific inferred from the complete DNA sequence of the mitochondrial control region. *Zool Stud* 45: 269–79.
- Lutz VA, Carreto JI. (1991). A new spectrofluorometric method for the determination of chlorophyll-a and degradation products and its application in two frontal areas of the Argentine Sea. *Continental Shelf Res* 11:433–51.
- Matano RP, Schlax MG, Chelton DB. (1993). Seasonal variability in the southwestern Atlantic. *J Geophys Res* 98:18027–35.
- Matano RP, Palma ED, Piola AR. (2010). The influence of the Brazil and Malvinas Currents on the Southwestern Atlantic Shelf circulation. *Ocean Sci* 6:983–95.

- Menezes NA, Buckup PA, Figueiredo JL, Moura RL. (2003). Catálogo das espécies de peixes marinhos do Brasil. São Paulo: Museu de Zoologia da USP.
- Militelli MI, Macchi GJ. (2004). Spawning and fecundity of king weakfish, *Macrodon ancylodon*, in the Rio de la Plata estuary, Argentina-Uruguay. *J Marine Biol Assoc United Kingdom* 84:443–7.
- Muto EY, Soares LSH, Rossi-Wongtschowski CLDB. (2000). Demersal fish assemblages of São Sebastião, southeastern Brazil: Structure and environmental conditioning factors (summer 1994). *Brazilian J Oceanography* 48:9–27.
- Nei M. (1987). *Molecular evolutionary genetics*. New York: Columbia University Press.
- Passos AC, Contente RF, Araujo CCV, Daros FALM, Spach HL, Abilhô V, Fávoro LF. (2012). Fishes of Paranaguá Estuarine Complex, South West Atlantic. *Biota Neotropica* 12:226–38.
- Piola A, Campos EJD, Moller Jr OO, Charo M, Martinez C. (2000). Subtropical shelf front off eastern South America. *J Geophysical Res* 105:6565–78.
- Piola AR, Matano RP. (2001). The South Atlantic Western Boundary Currents Brazil/Falkland (Malvinas) Currents. In: Steele JM, Thorpe SA, Turekian KK (Eds.), *Encyclopedia of ocean sciences*. London, UK: Academic Press, Vol. 1, pp. 340–9.
- Plavan AA, Passadore C, Gimenez L. (2010). Fish assemblage in a temperate estuary on the Uruguayan coast: Seasonal variation and environmental influence. *Brazil J Oceanography* 58:299–314.
- Posada D, Crandall KA. (2001). Intraspecific gene genealogies: trees grafting into networks. *Trends Ecol Evol* 16:37–45.
- Ramírez-Soriano A, Ramos-Onsins SE, Rozas J, Calafell F, Navarro A. (2008). Statistical power analysis of neutrality tests under demographic expansions, contractions and bottlenecks with recombination. *Genetics* 179:555–67.
- Rocha MLCF, Fernandez WS, Paiva-filho AM. (2010). Spatial and temporal distribution of fish in Palmas Bay, Ubatuba, Brazil. *Brazil J Oceanography* 58:31–43.
- Rodrigues R, Schneider H, Santos S, Vallinoto M, Sain-Paul U, Sampaio I. (2008). Low levels of genetic diversity depicted from mitochondrial DNA sequences in a heavily exploited marine fish (*Cynoscion acoupa*, Sciaenidae) from the Northern coast of Brazil. *Genet Molecular Biol* 31:487–92.
- Sambrook J, Russell DW. (2001). *Molecular cloning: A laboratory manual*. New York: Cold Spring Harbor laboratory Press.
- Sampaio I, Santos S, Schneider H. (2010). Molecular phylogenetic and population genetic structuring of *Macrodon* sp, an estuarine dependent Fish of the Western Atlantic Ocean. In: Saint-paul U, Schneider H, editors. *Mangrove dynamics and management in North Brazil*. Berlin, Germany: Spring Verlag.
- Santos EP, Yamaguti N. (1965). Migração da pescada foguete *Macrodon ancylodon*. *Boletim do Instituto Oceanográfico* 14:129–38.
- Santos S, Hrbek T, Farias IP, Schneider H, Sampaio I. (2006). Population genetic structuring of the king weakfish, *Macrodon ancylodon* (Sciaenidae), in Atlantic coastal waters of South America: Deep genetic divergence without morphological change. *Mol Ecol* 15: 4361–73.
- Santos S, Schneider H, Sampaio I. (2003). Genetic differentiation of *Macrodon ancylodon* (Sciaenidae, Perciformes) populations in Atlantic coastal waters of South America as revealed by mtDNA analysis. *Genet Mol Biol* 26:151–61.
- Schmidt TCS, Dias JF. (2012). Pattern of distribution and environmental influences on the Scienidae community of the Southeastern Brazilian coast. *Brazil J Oceanography* 60:233–43.
- Seyoum S, Tringali MD, Bert TM, Mcelroy D, Stokes R. (2000). An analysis of genetic population structure in red drum, *Sciaenops ocellatus*, based on mtDNA control region sequences. *Fishery Bull* 98: 127–38.
- Tajima F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585–95.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–9.
- Wang M, Zhang X, Yang T, Han Z, Yanagimoto T, Gao T. (2008). Genetic diversity in the mtDNA control region and population structure in the *Sardinella zunasi* Bleeker. *Afr J Biotechnol* 7:4384–92.
- Yamaguti N. (1968). Mortalidade da pescada-foguete, *Macrodon ancylodon*. *Boletim do Instituto Oceanográfico* 17:67–70.
- Yamaguti N. (1979). Diferenciação geográfica de *Macrodon ancylodon* (Bloch & Schneider, 1801) na costa brasileira, entre as latitudes 18o36'S e 32o10'S: etapa I. *Brazil J Oceanogr* 28:53–118.
- Yamaguti N, Santos EPD. (1966). Crescimento da pescada-foguete (*Macrodon ancylodon*): Aspecto quantitativo. *Boletim do Instituto Oceanográfico* 15:75–8.