Differences in haplotype frequencies of mtDNA of the Spanish sardine *Sardinella aurita* between specimens from the eastern Gulf of Mexico and southern Brazil

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Abstract. – Analysis of restriction-site polymorphisms of mtDNA and Monte Carlo statistics were used to test the hypothesis that at least two genetic stocks of Spanish sardine *Sardinella aurita* are present in the eastern Gulf of Mexico, with one occurring at each of the two main fishery locations off Florida. Also included for comparison were specimens of Spanish sardine collected off southern Brazil. No significant heterogeneity of mtDNA haplotype frequencies was detected among specimens from the two locations within the Gulf of Mexico after analysis of 57 individuals (28 from Tampa Bay and 29 from the Florida Panhandle) using 9 informative restriction enzymes. However, highly-significant differences were observed between the specimens from the Gulf of Mexico and 16 specimens from southern Brazil. Based on counts of gill rakers of all specimens from both regions and the results of the mtDNA analysis, it is suggested that *Sardinella brasiliensis*, the Brazilian sardine, is conspecific with *S. aurita*, and that *S. aurita* is probably represented by genetically-identifiable populations in the western North and South Atlantic.

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Spanish sardine *Sardinella aurita* (Valenciennes) ranges in the western Atlantic from Cape Cod, throughout the Gulf of Mexico and Caribbean Sea, to southern Brazil (Whitehead 1973). It is also present in the Mediterranean Sea and in the eastern Atlantic off the African coast (Fisher 1978, Whitehead 1985). A second sardine species, *Sardinella brasiliensis* (Steindachner), reportedly occurs within the range of *S. aurita* between the Gulf of Mexico and southern Brazil (Whitehead 1973 and 1985, Johnson & Vaught 1986), but there have been no studies successful in fully differentiating the two species within the eastern Gulf of Mexico (Wilson & Alberdi 1991). There has traditionally been a small but important baitfish fishery for Spanish sardine off Florida with an annual landing of over 1500 t as recently as 1988. However, this fishery closed abruptly in 1989 after landings dropped sharply between 1988 and 1989. The closure was partly a response to uncertainty about the number of species or stocks being fished (Sutter & Mahmoudi 1992).

Management concerns about the sardine fishery in the eastern Gulf of Mexico involve the possibility of local stock depletion or collapse from increased fishing pressure at the two primary fishing areas in the eastern Gulf of Mexico, the Florida Panhandle and Tampa Bay. There is also concern over the possibility of overfishing one of the two species supposedly present. The first concern has arisen from the observation that Spanish sardines caught by the fisheries operating at these two locations appear to consistently differ morphometrically (Johnson & Vaught 1986), suggesting the possibility of separate genetic stocks or species. In response to these concerns, Wilson & Alberdi (1991) electrophoretically compared more than 300 specimens from the two areas at 41 presumptive genetic loci and found no important differences in allele frequencies, nor any fixed differences. They concluded that stock differences were only weakly indicated, and that no genetic evidence of two species of Spanish sardine in the eastern Gulf of Mexico had appeared.

Analysis of restriction-site polymorphisms of mitochondrial DNA (mtDNA) is generally regarded as offering a higher degree of resolution than protein electrophoresis in population genetic studies (e.g., Ferris & Berg 1987), and has recently become important in studies of potential fishery stocks. The most direct analytical approach is to compare the frequency distributions of composite mtDNA haplotypes among the sample populations, testing (x^2) whether the distributions are homogeneous and, therefore, indicative of stock mixing (Bentzen et al. 1989). However, the usually skewed distribution toward a common haplotype(s) coupled with the common occurrence of several rare haplotypes (Billington & Hebert 1991) in a population sample usually results in low sample numbers per cell in contingency tables, and tabulated values of x^2 cannot be used properly. This problem can be avoided, however, by using a Monte Carlo method of computing the exact x^2 expected from the observed haplotype frequencies among sample populations (Roff & Bentzen 1989, Bernatchez & Dodson 1990).

In this study we employ analysis of restriction-site polymorphisms of mtDNA and Monte Carlo statistics to test the hypothesis that the two main fisheries in the eastern Gulf of Mexico are comprised of at least two genetic stocks of *S. aurita*. We have included specimens of *S. aurita* collected off southern Brazil under an *a priori* expectation that stronger differences in mtDNA haplotype frequencies should exist between specimens from the Gulf of Mexico and southern Brazil. Considering the geographic proximity of our sample populations in the eastern Gulf of Mexico, and recognizing the fact that inadequate sampling of the mitochondrial genome (as evidenced by the number of restriction enzymes used) can bias the outcome in the direction of "no significant differences," inclusion of the Brazilian specimens allows us to objectively gauge the power of our methods to address the stated hypothesis.

Materials and methods

Specimens of *S. aurita* from the eastern Gulf of Mexico were obtained from the two main commercial fisheries along Florida's west coast, one near Tampa Bay and the other off the Panhandle. Individuals were collected fresh from both sites during the summer of 1989 (Fig. 1). Ripe ovaries were excised soon after capture and held on ice or in refrigerated buffer (MSB-Ca^{++} of Lansman et al. 1981) for up to 10d prior to purification of mtDNA; some ovaries from the Tampa Bay sample were stored frozen at –86°C until processing. The timing of specimen collections was structured to avoid resampling a single migrating school. Two samples were taken concurrently from Port St. Joe, Florida (n=10) and Destin, Florida (n=13) in May 1989, and a third sample was taken from Destin (n=6) in September 1989, for a total of 29 specimens (Fig. 1). In the Tampa Bay region a total of 28 individuals were taken during three collection times: February (n=12), May (n=10), and August (n=6) of 1989.

Whole fish specimens obtained from off Santos, Brazil were collected from the fishery and hand-carried frozen on dry ice to St. Petersburg, Florida within 2d and were stored at –86°C for several months without removing the ovaries. Because these specimens were

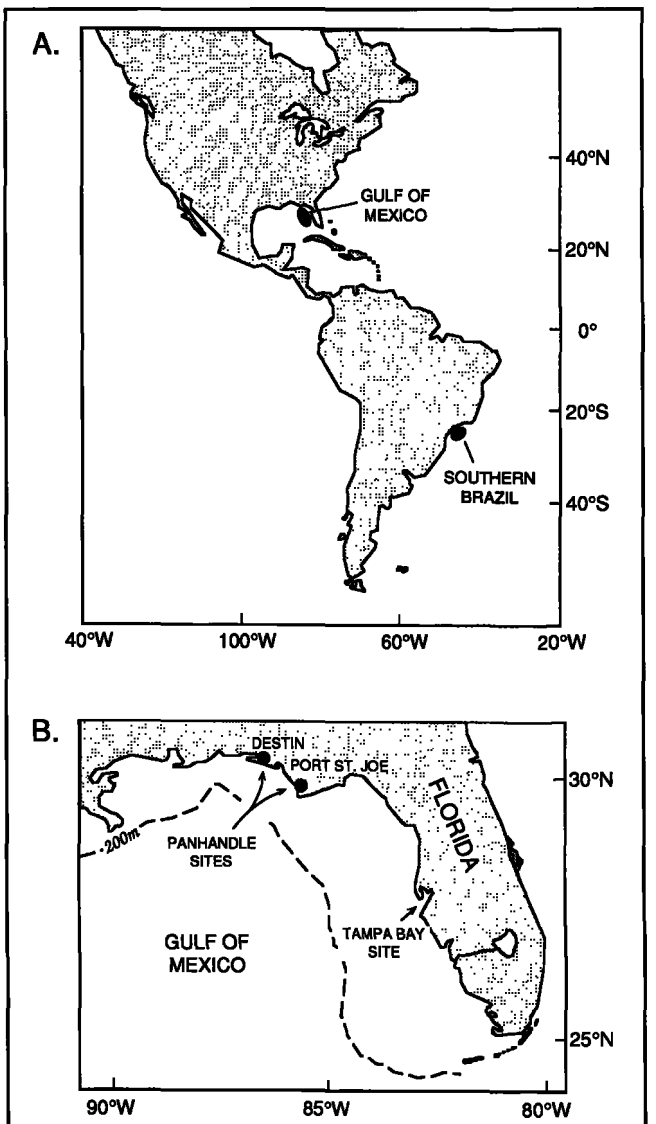

Figure 1

Collection sites of Spanish sardines *Sardinella aurita* in the eastern Gulf of Mexico and southern Brazil: **(A)** The two major sites, and **(B)** two area sites in the eastern Gulf of Mexico.

also used in the electrophoretic study of Wilson & Alberdi (1991), one thaw/refreeze cycle occurred prior to use in the present study. All specimens were identified to nominal species using the criteria of Whitehead (1973, 1985). That is, we counted the number of gill rakers on the lower limb of the first gill arch and related the count to standard length (SL), and we examined the condition (curled or flattened) of the anterior rakers of the lower limb of the first arch.

Mitochondrial DNA was purified from the ripe ovaries of the specimens taken from the Gulf of Mexico as in Wilson & Tringali (1990), except that the mtDNA collected after density-gradient ultracentrifugation was extracted in n-butanol which was not saturated with NaCl. This removed most of the salts, allowing greater precision over the molar concentration of the DNA-containing solution during the ethanol precipitation. The lower yield of mtDNA caused by thawing and refreezing of the Brazilian specimens necessitated use of a modification of the Chapman & Powers (1984) method of extraction (Tringali 1991).

Restriction digests were carried out as specified by the manufacturers using the following enzymes: *Apa*-I, *Bam*H-I, *Dra*-I, *Eco*R-I, *Hin*d-III, *Pst*-I, *Pvu*-II, *Sac*-I, *Xba*-I, and *Xho*-I. Fragments were separated by horizontal agarose gel electrophoresis and visualized in most cases using the gel-incorporated Hoechst 33258 (CalBiochem #SR5A03-0388) fluorochrome dye as in Deflaun & Paul (1986). However, some poor yields of mtDNA during purification made visualization of the smaller restriction fragments difficult using the fluorochrome, thus requiring application of an mtDNA hybridization probe to southern blots of the agarose gels (Tringali 1991). The non-radioactive hybridization probe was made from cesium-purified mtDNA of *S. aurita* using a commercial kit (Genius Kit of Boehringer Mannheim, Cat. #1093657). Hybridization and immunological detection of the probe was performed as specified by the manufacturer with the following modifications. The concentration of the blocking reagent in the prehybridization and hybridization solutions was doubled to 1.0% weight/volume to reduce background coloring of the hybridization filter (Zeta-Probe). The prehybridization period was extended to a minimum of 2h and the volume of the probe-containing hybridization solution was increased to 10 m L/100 cm^2 of filter. The ELISA reaction period was increased to 2 h. Under these modifications, the mtDNA restriction fragments were usually visible on the filter ~15 min into the color reaction.

Molecular weights (in base pairs) of the restriction fragments were estimated by the global form of the reciprocal method as described in Elder & Southern (1987). Restriction-fragment length polymorphisms (RFLPs) were expressed as two data types: restriction-site data and composite mtDNA haplotypes. The restriction-site composition of each individual was inferred from fragment profiles and intraspecific sequence diversity (Nei & Miller 1990) was calculated using the RESTSITE v1.2 algorithm. Composite mtDNA haplotypes were constructed for each individual from their restriction profiles with haplotypes grouped by sample location and time of collection. The frequency distribution of mtDNA haplotypes was tested for heterogeneity, with respect to location and time, by the log-likelihood G-test for independence (Sokal & Rohlf 1981). G-tests were also performed on selected samples following generation of 100 randomized data sets from the observed data, using the *Monte* Carlo technique described by Roff & Bentzen (1989) where the probability, P, of obtaining a randomized G_H value greater than the original observed value is given by

$$P = n / N_r \quad (1)$$

where n equals the number of outcomes in which the randomized G_H value is greater than the actual G_H value, and N_r represents the number of randomizations performed. The standard error of P was taken as

$$[P(1-P)/N_r]^{1/2}. \quad (2)$$

The frequency of the most common haplotype was tested, after arcsine square-root transformation (Freeman & Tukey 1950), using the "V" statistic (Desalle et al. 1987) for heterogeneity between sampling locations.

Results

Counts of gill rakers identified 72 of 73 sample specimens as *S. aurita*; one specimen from Brazil had gill-raker counts expected for *S. brasiliensis*. Counts were lowest for the Panhandle specimens while specimens from Tampa Bay and southern Brazil were the most similar, having higher counts (Table 1). Specimens from the Florida Panhandle were of larger average size than those from Tampa Bay and southern Brazil, which were more similar in size (Table 1). A one-way analysis of variance revealed significant heterogeneity ($P<0.05$) in gill-raker counts between the specimens taken from the Panhandle and Tampa Bay, but not between those taken from Tampa Bay and southern Brazil. One Brazilian specimen had a gill-raker count of 154 at 150 mm SL, nearly identical to that reported for the lectotype of *S. brasiliensis* (155 at 148.3 mm SL; Whitehead 1973). However, we could detect no differences in the appearance of the anterior rakers (curled vs. flattened) for this or other specimens. The overall appearance of the rakers of all specimens most closely resembled Whitehead's (1985) diagrammatic drawing

Table 1
Data for Spanish sardines *Sardinella aurita* used in mtDNA analysis.

Location	Number counted	Size range[1] (mmSL)	gill-raker count Mean[2]	gill-raker count Range
Port St. Joe (Panhandle)	10	162–189	113±11SD	102–127
Destin (Panhandle)	19	159–189	114±9	99–128
Tampa Bay	28	149–171	134±7	121–145
Santos, Brazil	16	149–181	132±9	122–154

[1] Mean length of pooled specimens from the Panhandle is significantly larger than that of specimens from the other two regions (ANOVA, $P<0.05$).
[2] Mean number of gill rakers of pooled specimens from the Panhandle is significantly lower than that of specimens from the other two regions (ANOVA, $P<0.05$).

of *S. brasiliensis*, curling anteriorly downward. However, use of this character was extremely problematic for us because, other than the drawing, we found no published taxonomic account of the discovery of this character, no photographic evidence nor written description detailing the character, nor reference of this character to [any] catalogued or type material.

The combination of mtDNA purification and visualization techniques used here provided well-resolved digestion patterns that could be consistently and unambiguously scored for all individuals. Of the 10 restriction endonucleases used, only *Bam*H-I was not informative. This enzyme had but one restriction site in all samples and was used only in probe construction to linearize template mtDNA prior to labeling. Polymorphic digestion patterns occurred for the remaining nine enzymes, with *Pst*-I and *Pvu*-II highly variable (Table 2). The mean mtDNA genome size, calculated from the sums of all digestion patterns, was 16,304 base pairs (bp) ± 71 bp (SD). No size variation was detected between specimens or within the mtDNAs of individual specimens.

The nine informative enzymes sampled 52 unique restriction sites over all individuals (Table 3) with a mean of 43 sites per individual. All RFLPs were consistent with the assumption of a single nucleotide substitution between variant digestion patterns. Intraspecific sequence diversity (Nei & Miller 1990) over all individuals was 0.00525±0.00204 (bootstrapped SE) substitutions per nucleotide, a value consistent among all three sampling locations ($\bar{x}$=0.00509±0.00054, range 0.00452–0.00561). Twenty-four composite mtDNA haplotypes were generated from polymorphic restriction-fragment profiles of the 73 completely characterized Spanish sardines (Table 4). The composite mtDNA haplotype (BAABAAAAA) of the one putative *S. brasiliensis* specimen with a gill raker count close to the lectotype's (Whitehead 1970, 1973) was identical to that of another Brazilian specimen having the low gill raker count indicative of *S. aurita* (126 at 169 mm SL), as well as to that of seven specimens from the Gulf of Mexico (Table 4). The mtDNA haplotype most common in the Gulf of Mexico, AAAAAAAAA, occurred in very low frequency among the Brazilian specimens. The frequency of this haplotype was significantly different between specimens of the Gulf of Mexico and Brazil ($V=10.90$, $P<0.001$), but not among specimens from the different regions within the Gulf of Mexico (Table 5).

Ten composite haplotypes were unique to the Panhandle region, four to the Tampa Bay region, and five to Brazil. Four were observed in all three locations. G-tests of mtDNA haplotype frequencies for temporal and geographical heterogeneity among specimens from within the Gulf of Mexico did not allow rejection of the null hypothesis of population homogeneity ($P>0.05$). Pooling the Gulf specimens by location and testing for heterogeneity between locations using Monte Carlo statistics (100 randomizations) generated 35 G_H values exceeding the initial value of 25.774, again indicating that the null hypothesis of homogeneity for specimens taken within the Gulf of Mexico cannot be rejected ($P>0.35$, 2SE=0.09). Pooling all Gulf specimens and testing against the Brazilian specimens using Monte Carlo statistics resulted in a G_H value of 44.282 which was not exceeded by any of the 100 randomizations, indicating significant heterogeneity between specimens from those locations ($P<0.01$).

Discussion

Our results indicate an evident lack of genetic stock structuring between the commercially-fished populations of Spanish sardine in the eastern Gulf of Mexico, even though there is a significant difference in the average number of gill rakers between locations, in addition to the morphometric differences reported by Johnson & Vaught (1986). This significant variation in gill-raker counts relative to SL appears to confirm Whitehead's (1973) reference to two rates of increase in the number of gill rakers with increasing standard length among Spanish sardines of the western Atlantic, although the lowest counts (Panhandle) were lower than those Whitehead (1973) mentioned. Whitehead (1973) thought this variation in gill-raker counts indicative of the presence of two valid species of Spanish sardines in the western Atlantic, but our mtDNA analysis does not support his suggestion.

Table 2

Approximate sizes (base pairs) of restriction fragments from mtDNA of *Sardinella aurita*. Letters designate genotypes observed with each enzyme. "A" genotype was the most common for each enzyme.

Apa-I		*Bam*H-I	*Dra*-I		*Eco*R-I		*Hin*d-III		
A	B	A	A	B	A	B	A	B	C
6457	6457	16356	10233	8434	5621	5621	4169	3342	6607
7405	7405		5623	5623	4069	4069	3342	3296	4169
1948	1948		438	1798	3436	3436	3296	2661	2661
360	460			438	1318	1318	2661	2251	2104
107					665	1147	2104	2104	436
					620	665	436	1929	331
					525		331	436	
								331	
16277	16270	16356	16294	16293	16254	16256	16339	16350	16308

Pst-I							*Xho*-I		
A	B	C	D	E	F	G	A	B	C
4880	7100	7100	4360	4880	8184	4880	10965	9593	16274
4360	4360	4880	4200	4360	4880	4360	5309	5309	
3850	4200	4360	3850	3800	3150	3850		1372	
3150	564		3150	3000		2350			
			564	150		750			
16249	16224	16340	16124	16190	16214	16190	16274	16274	16274

Pvu-II								*Sac*-I	
A	B	C	D	E	F	G	H	A	B
6461	6461	10133	10133	6461	6461	6461	10753	7786	15230
4265	3672	2238	2238	3672	4770	3672	2238	7444	912
2238	2238	1779	1779	2238	2238	1878	1778	912	
1778	1778	1188	912	1779	1779	1778	912		
912	1188	912	690	912	912	1188	505		
505	912	230	505	690	230	912	230		
230	230		230	505		360			
				230		230			
16389	16479	16480	16487	16487	16390	16479	16416	16142	16142

Xba-I	
A	B
8317	6905
4809	4809
	2248
2248	1402
685	685
335	335
16394	16384

The intraspecific sequence variability of mtDNA of *S. aurita* is comparable to that observed in most other marine fishes. With a mean sequence divergence of about p=0.51 (Nei & Li 1979), the mtDNAs of Spanish sardines of the Gulf of Mexico fall within the range of mean values observed among other clupeids, i.e., between 0.37% for shad (Bentzen et al. 1989) and 2.4% for menhaden (Avise et al. 1989). In both shad (Roff & Bentzen 1989) and menhaden (Bowen & Avise 1990), population-level mtDNA variability was observed

Table 3

Site matrices inferred from RFLPs of *Sardinella aurita*. Letters denote restriction patterns. Column numbers are the restriction sites observed over all individuals for the given enzyme. Inside the matrices: 1=site presence, 0=site absence.

	Pst-I							*Xba*-I						*Hind*-III							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A	1	0	1	1	1	0	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1
B	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C	1	0	1	1	0	0	0							1	0	1	0	1	1	1	1
D	1	1	1	1	1	0	0														
E	1	0	1	1	1	0	1														
F	1	0	1	0	1	0	0														
G	1	0	1	1	1	1	0														

	Pvu-II									*Dra*-I				*Apa*-I				
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
A	1	1	0	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
B	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	0
C	1	0	1	0	1	1	1	1	0									
D	1	0	1	1	1	1	1	1	0									
E	1	1	1	1	1	1	1	1	0									
F	1	1	0	0	1	1	1	1	0									
G	1	1	1	0	1	1	1	1	1									
H	1	0	0	1	1	1	1	1	0									

	*Eco*R-I							*Xho*-I			*Sac*-I		
	40	41	42	43	44	45	46	47	48	49	50	51	52
A	1	1	1	1	1	1	1	1	0	1	1	1	1
B	1	1	1	1	1	0	1	1	1	1	1	0	1
C								1	0	0			

within the geographic regions sampled. Thus, the evident lack of significant heterogeneity in mtDNA haplotype frequencies among the Gulf specimens of *S. aurita* is probably not due to an inadequate level of mtDNA genome sampling relative to the sequence variability present.

Accepting that the mtDNA variability among sample locations of *S. aurita* was sufficiently characterized, at least two possibilities could account for the observed genetic homogeneity between our sample locations in eastern Gulf of Mexico. This lack of genetic structuring is the result of recent or ongoing genetic exchange between the sampled locations, or there may have been insufficient time for discrete Gulf populations, if they do exist, to diverge through mtDNA lineage sorting. The first explanation seems the more likely one, considering the life history of the species. *S. aurita* is known to be highly migratory (Hildebrand 1963, Simpson & Gonzalez 1967) and to spawn repeatedly throughout much of the year (Grall 1984). Although the pattern of adult migration of *S. aurita* in the eastern Gulf is still poorly understood, spawning occurs continuously between the Panhandle and Tampa Bay with no apparent geographic partitioning in either the distribution or abundance of eggs or larvae (Houde 1976). There are no known physical barriers to dispersal or migration in this region.

Significant geographic variation in mtDNA haplotype frequencies does exist between Spanish sardines in the eastern Gulf of Mexico and waters off southern Brazil, based upon the results of goodness-of-fit testing. This variation is due to two related factors. One is that the haplotype most common among the pooled specimens from the eastern Gulf of Mexico, AAAAAAAAA, possessed by 46% of the specimens, has a much lower occurrence (6%) among the specimens from southern Brazil. The other is the difference in the distribution of the *Pst*-I digestion pattern. Whereas 5 of 16 (31%) of the Brazilian specimens possessed the G-type pattern (Table 2), this pattern was absent in the Gulf specimens (n=57). Relative to the common A-type pattern of *Pst*-I present among the Gulf specimens, this G-type pattern represents a site gain at *Pst*-I.

Table 4

Frequency distribution of the 24 composite haplotypes from 73 *Sardinella aurita* from the western Atlantic. Restriction enzymes are in the following order: *Pst*-I, *Xba*-I, *Hin*d-III, *Pvu*-II, *Dra*-I, *Apa*-I, *Eco*R-I, *Xho*-I, and *Sac*-I. Frequency distribution of pooled haplotypes from the Gulf of Mexico differ from the Brazilian at $P<0.01$ (G_H=44.282).

Haplotype number	Composite digestion profile	Panhandle Florida	Tampa Bay Florida	Santos Brazil
1	AAAAAAAAA	14	12	1
2	BAABAAAAA	1	6	2*
3	AAADABAAA	1		
4	AABAAAAAA	1		
5	BAACBAABA	1		
6	BBABAAAAA	1		
7	BAACAAAAA	2	1	
8	CAAAAAAAA	1		
9	DAAAAABAA	1		
10	BAAFAAAAA	1		
11	CAABAAAAA	1	4	3
12	AAAAAAACA	1		
13	AAAEAAAAA	1	1	1
14	EAAAAAABA	1		
15	AAAEABBAA	1		
16	BAABAAAAB		1	
17	FAAAAAAAA		1	
18	BAAGAAAAA		1	
19	AAAFAAAAA		1	
20	AAAHAAAAA			2
21	GACAAAAAA			2
22	GAAAAAAAA			3
23	BAAAAAAAA			1
24	AAAAABAAA			1
		29	28	16

*Including specimen identified as *S. brasiliensis* by gill-raker count.

Table 5

Tests for temporal and geographic heterogeneity of *Sardinella aurita* mtDNA haplotypes. G_H values were generated by comparing the complete distribution of mtDNA haplotypes for each group. "V" statistics were calculated by comparing the frequency of angular-transformed Haplotype 1 between regions.

Sample	G_H	df	V	df
Tampa Bay vs. Panhandle	24.278	17	0.01	1
Tampa Bay vs. Brazil	29.302*	12	9.68*	1
Panhandle vs. Brazil	39.386*	19	11.49**	1
Pooled Gulf of Mexico vs. Brazil	44.282*	23	10.90**	1

* $P<0.05$, ** $P<0.001$ (from tabulated critical values of the x^2 distribution).

We interpret the significant heterogeneity in haplotype frequencies between specimens from the eastern Gulf of Mexico and southern Brazil as genetic stock differences between Spanish sardines of the western North and South Atlantic, rather than as species differences as, for example, between *S. aurita* and *S. brasiliensis*. The one specimen of this study referable to *S. brasiliensis*, with a gill-raker count of 154 at 150 mmSL, came from southern Brazil, but it had one of the mtDNA composite haplotypes (haplotype 2; Table 4) present among specimens from the eastern Gulf of Mexico. All other specimens from both Gulf and Brazilian waters possessed the lower gill-raker counts typical of *S. aurita*.

This interpretation also agrees with results of the electrophoretic study of Wilson & Alberdi (1991). After comparing 350 specimens from the eastern Gulf of Mexico with 41 specimens of Spanish sardine from off southern Brazil at 36 presumptive gene loci, they found no genetic evidence of more than one species of Spanish sardine in those two areas. That is, there were no fixed allelic differences that would suggest the presence of fully segregated gene pools, as in two species. Neither in the present study nor in that of Wilson & Alberdi (1991), which used the same Brazilian specimens as here, was our nominal *S. brasiliensis* distinguishable from *S. aurita* by electrophoretic genotype or mtDNA haplotype.

We think it likely that *S. brasiliensis* is conspecific with *S. aurita*. Whitehead (1970) removed *S. brasiliensis* from the synonymy of *S. aurita* on the observation that the syntypes of *S. brasiliensis* from off Brazil had both high and low gill-raker counts at similar lengths. That is, he concluded that the syntypes with low gill-raker counts were *S. aurita* and that the others with high gill-raker counts were *S. brasiliensis* of Steindachner. Whitehead (1973) again proposed the specificity of *S. brasiliensis* on this character based on his review of published data on gill-raker counts of western Atlantic *Sardinella*, but he did not report data from any additional specimens. Whitehead (1985) proposed that *S. brasiliensis* might be distinguished from *S. aurita* based on the shape of the gill rakers forming the branchial basket, i.e., flattened in *S. aurita* vs. curled in *S. brasiliensis*, but he did not specifically connect this character to catalogued specimens which he examined. No mention was made of this character in his prior discussion of the syntypes (Whitehead 1970).

To our knowledge, no thorough quantitative study of variation in gill-raker counts with SL among Brazilian sardines has been published. Montero & Perez

(1981) conducted a protein electrophoretic comparison at 24 presumptive loci of Brazilian *Sardinella* which had been first identified as either *S. brasiliensis* or *S. aurita* by high or low gill-raker counts, respectively. They reported no electrophoretic differences between the two putative species, which is consistent with our finding of significant variation in gill-raker counts between specimens from the Florida Panhandle and Tampa Bay in the apparent absence of any genetic differences (Table 1). Thus, the higher gill-raker counts which supposedly distinguish *S. brasiliensis* from *S. aurita* are probably due to non-genetic causes, and are of no greater taxonomic importance than the intraspecific variation in gill-raker counts observed between *S. aurita* inhabiting the Panhandle and Tampa Bay locations of the eastern Gulf of Mexico (Table 1). Until the present classification of Western Atlantic *Sardinella* is supported by a thorough study of variation in the characters on which it is based, the validity of *S. brasiliensis* as a species is in question.

Given the non-reticulate nature of mtDNA transmission, it is not difficult to understand intraspecific divergence when there is a mechanism between populations affecting the balance between gene flow and genetic drift. There are a number of potential barriers to gene flow within the trans-equatorial range of *S. aurita*, including diverging oceanic currents (e.g., the North Equatorial current) and the major river plume of the Orinoco which can extend far out into the Caribbean. Even without discrete physical barriers, the stochastic process of mtDNA lineage survival or extinction might in itself be enough to alter haplotype frequencies in this continuously-distributed species if the effective gene flow is low relative to the sizes of the populations or their geographic ranges (Neigel & Avise 1986). However, the mtDNA variability among *S. aurita* might be a product of differential selective forces because the species is distributed over so great a latitudinal (environmental) gradient. For the Spanish sardine of the western Atlantic, there are not yet enough data to choose between the alternative hypotheses of drift vs. selection in accounting for the evident genetic structuring.

To our knowledge, this report represents the first substantiated case of a holopelagic teleost with a supposedly continuous distribution between sample regions (Gulf of Mexico to southern Brazil) exhibiting significant heterogeneity in mtDNA haplotype frequencies. Whereas other studies (e.g., Graves & Dizon 1989) have compared sample populations of holopelagic fishes removed by great longitudinal distances, or on opposite sides of land masses (Ovenden et al. 1989), ours is the first to compare specimens at near-opposite ends of a trans-equatorial distribution spanning many degrees of latitude (30°N to 26°S). Not only do these results further confirm the potential usefulness of mtDNA in studies of hypothesized fishery stocks, but also suggest the need for additional studies on fishes having similar distributions.

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Citations

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