



**Phylogenetic Systematics of the *Scomberomorus regalis* (Teleostei: Scombridae)
Species Group: Molecules, Morphology and Biogeography of Spanish Mackerels**

Heidi M. Banford; Eldredge Bermingham; Bruce B. Collette; S. Shawn McCafferty

Copeia, Vol. 1999, No. 3. (Aug. 2, 1999), pp. 596-613.

Stable URL:

<http://links.jstor.org/sici?sici=0045-8511%2819990802%293%3A1999%3A3%3C596%3APSOTSR%3E2.0.CO%3B2-C>

Copeia is currently published by American Society of Ichthyologists and Herpetologists.

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/about/terms.html>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/asih.html>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

The JSTOR Archive is a trusted digital repository providing for long-term preservation and access to leading academic journals and scholarly literature from around the world. The Archive is supported by libraries, scholarly societies, publishers, and foundations. It is an initiative of JSTOR, a not-for-profit organization with a mission to help the scholarly community take advantage of advances in technology. For more information regarding JSTOR, please contact support@jstor.org.

Phylogenetic Systematics of the *Scomberomorus regalis* (Teleostei: Scombridae) Species Group: Molecules, Morphology and Biogeography of Spanish Mackerels

HEIDI M. BANFORD, ELDREDGE BERMINGHAM, BRUCE B. COLLETTE, AND
S. SHAWN MCCAFFERTY

Phylogenetic and historical biogeographic hypotheses were explored and inferred for the *Scomberomorus regalis* species group, composed of five New World species (*S. regalis*, *S. brasiliensis*, *S. maculatus*, *S. concolor*, and *S. sierra*) and the eastern Atlantic *S. tritor*, in testing the generalization of Rosen's eastern Pacific/eastern Atlantic track hypothesis. The Indo-West Pacific *S. guttatus* and Atlantic *S. cavalla* served as outgroups. The entire ATP synthase 8 [ATPase 8; 168 base pairs (bp)] and 6 (ATPase 6; 684 bp), and NADH ubiquinone oxidoreductase subunit 2 (ND2; 1047 bp) mitochondrial genes, and an approximately 400-bp portion of the nuclear encoded Aldolase exon 5/intron 5 were sequenced. The monophyly of the *S. regalis* species group was fully supported, with the eastern Atlantic *S. tritor* basal to the New World taxa as predicted from the eastern Atlantic/eastern Pacific generalized biogeographic track. Surprisingly, no differences were observed between the mitochondrial genomes of *S. regalis* and *S. maculatus*. However nuclear DNA and morphological characters confirm the species status of these two taxa, leading us to posit the introgressive loss of the *S. maculatus* mtDNA genome following hybridization with *S. regalis*. Chronological estimates were applied to the phylogenetic topology combining geologic dates for putative vicariant events with molecular distance values. These estimates indicate a pre-isthmian divergence for the western Atlantic *S. maculatus*, followed by divergence of the common ancestors of the western Atlantic (*S. brasiliensis* and *S. regalis*) and eastern Pacific (*S. concolor* and *S. sierra*) species resulting from the rise of the Isthmus of Panama and closure of associated seaways.

HISTORICAL biogeography seeks to reconstruct organismal distributions both spatially and chronologically. The identification of generalized tracks (L. Croizat, 1958, unpubl.), whereby common geographic and phylogenetic patterns are observed across diverse groups of taxa, is a primary objective of the reconstructive process. One marine biogeographic pattern of general interest is the eastern Pacific/eastern Atlantic track (EP/EA; Rosen, 1975), extending from the eastern Pacific across the Central American Isthmus to the Caribbean, with a transatlantic connection to the West African coast. This track has two components, the first is the transatlantic faunal connection (Metzelaar, 1919), with possible origins in the Cenozoic opening of the south Atlantic Ocean. The second component is the well-known New World transisthmian track. The isthmian completion offers a vicariant hypothesis for marine faunal relationships between the eastern Pacific and western Atlantic, based on a broad foundation of biological (Jordan, 1908; Vawter et al, 1980; Bermingham and Lessios, 1993; and numerous others) and earth history studies (Coates et al, 1992; Coates and Obando, 1996;

Duque-Caro, 1990a, 1990b). The EP/EA track represents the geographic distribution of a number of putatively monophyletic fish taxa (Collette and Russo, 1981; Helfman et al., 1997), thus providing extensive opportunity for evaluating models of marine species diversification. Herein we provide the first phylogenetic assessment of the diversification model(s) implied by the EP/EA track using one such taxon, the *Scomberomorus regalis* species group (Fig. 1). A comprehensive evaluation of the model(s) will require phylogenetic hypotheses for additional monophyletic species groups distributed across the EP/EA track. Investigations of other groups hypothesized to share this track, such as the Belonidae and Hemiramphidae, are currently underway.

The scombrid genus *Scomberomorus* contains 18 nominal species ranging worldwide in tropical to temperate latitudes. Most species are coastal epipelagic and restricted to a portion of an ocean basin or sea by the 20 C isotherm. Though pelagic, adults generally do not undertake long open-ocean migrations as evidenced by their restricted species ranges. Six monophyletic clades or species groups are hypothesized

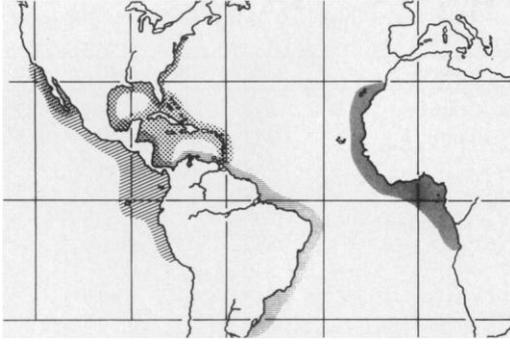


Fig. 1. The geographic distribution of the six species in the *Scomberomorus regalis* species group: *S. brasiliensis* (wavy lines), *S. concolor* (small stipple), *S. maculatus* (cross-hatch), *S. regalis* (large stipple), *S. sierra* (heavy diagonal), *S. tritor* (fine diagonal).

within the genus. The wahoo, *Acanthocybium solandri*, is considered the sister taxon (Collette and Russo, 1985b). The *Scomberomorus regalis* species group is united by the presence of nasal denticles and is the most derived clade in the genus (Collette and Russo, 1985b). It comprises six species, eastern Atlantic *S. tritor* and five New World species, eastern Pacific *S. sierra* and *S. concolor*, and western Atlantic *S. brasiliensis*, *S. maculatus*, and *S. regalis* (Collette and Russo, 1985b).

Converting Collette and Russo's (1985a, 1985b) phylogenetic hypothesis for the *S. regalis* clade (Fig. 2) into an area cladogram, encompassing both transatlantic and transisthmian tracks, provides a preliminary biogeographic hypothesis. However, relationships within this group are based on a small number of morphological characters (Fig. 2). A molecular phylogeny for the *S. regalis* species group provides an independent test of the Collette and Russo (1985b) hypothesis. In turn, we present a more robust phylogeny of the group as well as genetic distance data, permitting a spatial and temporal evaluation of the EP/EA biogeographic track. Our phylogenetic hypothesis is based on three complete genes totaling approximately 1900 bp of the mitochondrial genome, a nuclear intron of approximately 400 bp, and the morphological data of Collette and Russo (1985b). MtDNA and nuclear-encoded molecular markers provide independent measures of both the pattern and relative chronology of diversification (Vawter and Brown, 1986; Bermingham and Lessios, 1993; Bermingham et al., 1997).

MATERIALS AND METHODS

Specimens of each species were collected from different locations in an attempt to rep-

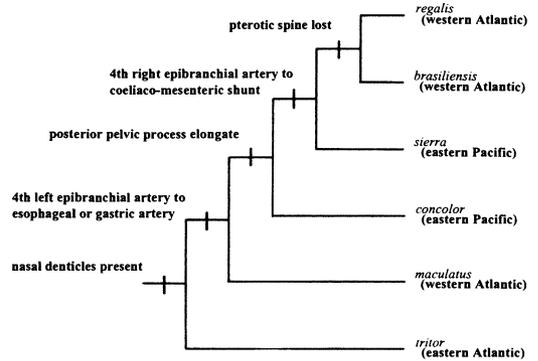


Fig. 2. Relationships within the *Scomberomorus regalis* group based on morphology, with morphological synapomorphies indicated (after Collette and Russo, 1985b).

resent variation across the geographic ranges (Fig 1). Gill and or muscle tissue was removed from either fresh or frozen specimens and stored in salt saturated DMSO/EDTA buffer (Seutin et al., 1990) at 4 C. After tissue removal, fish were preserved in formalin or maintained frozen as voucher specimens. Specimens used in this study have been accessioned at the United States National Museum of Natural History (USNM), Virginia Institute of Marine Science (VIMS), and Smithsonian Tropical Research Institute (STRI; Table 1). Genbank Accession numbers are provided in Appendix 1.

Genomic DNA was isolated by digesting approximately 0.2 g of gill or muscle tissue, rinsed with ddH₂O to remove salt, in 500 μ l of 2X CTAB buffer (1M Tris, 4M NaCl, 0.5M EDTA, CTAB, 2-Mercaptoethanol, pH 8.0; Saghai-Marouf et al., 1984) with 10 μ l of 100 mg/ml proteinase K and incubated at 56 C for 2–4 h with rotation, followed by phenol-chloroform-isoamyl alcohol (PCI; 25:24:1 by volume) and chloroform-isoamyl alcohol (CI; 24:1) extraction. Samples were dialyzed at 4 C in 1X TE buffer (10mM Tris, 1mM EDTA, pH 7.5) for 24–48 h. Genomic DNA samples were archived at –70 C; working aliquots were maintained in the laboratory at –20 C.

Five gene regions, the mitochondrial ATP synthase 8 and 6 (ATPase 8, 6; Nagley, 1988; Futai et al., 1989), NADH ubiquinone oxidoreductase subunit 2 (ND2; Ragan, 1987) and 12S ribosomal subunit genes, and nuclear Aldolase intron 5 (ALD; Lessa and Applebaum, 1993), were chosen for sequencing based on their potential to provide phylogenetic signal at different divergence times. Specifically the mtDNA protein coding genes, ATPase 8,6 and ND2 have been applied in a wide range of studies in our

TABLE 1. *Scomberomorus* MATERIAL SEQUENCED, NUMBER OF BASE PAIRS (bp) LISTED FOR EACH GENE REGION.

Species	Catalog #	Spec. ID#	Location	ATP8, 6	ND2	ALD	12s
<i>regalis</i>	USNM343341	HB872	Colombia (Atlantic)	842	1,047	408	425
	STRI-3836	STRI3836	Panama (Atlantic)	842	1,047	408	425
	USNM343344	STRI3973	Bahamas	842			425
	USNM343355	STRI3977	Bahamas	842			425
<i>brasiliensis</i>		HB700	Brazil	842			
		HB701	Brazil	842			
		HB702	Brazil	842			
		HB704	Brazil	842	1,047	408	
		HB705	Brazil	842			
	STRI-4276	STRI4276	Trinidad	842			
	STRI-4277	STRI4277	Trinidad	842	1,047	408	
<i>sierra</i>	USNM343347	HB857	Colombia (Pacific)	842	1,047	408	
	STRI-HB948	HB948	Panama (Pacific)	842			
	STRI-HB1191	HB1191	Panama (Pacific)	842	1,047	408	
<i>concolor maculatus</i>	USNM344347	HB966	Mexico (Gulf of California)	842	1,047	408	
	VIMS9587	HB260	U.S. (Atlantic)	842			425
	VIMS9588	HB261	U.S. (Atlantic)	842	1,047	407	425
	VIMS9591	HB264	U.S. (Atlantic)	842	1,047	407	425
	VIMS9592	HB265	U.S. (Atlantic)	842	1,047	407	425
	VIMS9593	HB266	U.S. (Atlantic)	842			425
		STRI5090	U.S. (Gulf of Mexico)	842	1,047		425
		STRI5093	U.S. (Gulf of Mexico)	842	1,047		425
<i>tritator</i>		HB968	Africa (Atlantic)	842	1,047	391	
		STRI4017	Angola	842	1,047	391	
<i>guttatus cavalla</i>	USNM347822	STRI4720	Philippines	842	1,047	356	
		HB803	U.S. (Gulf of Mexico)	842	778	410	

lab to examine both marine separations and terrestrial connections and dispersal resulting from the isthmian uplift (Bermingham et al., 1997). We included multiple mtDNA genes and a gene region (12S) for two reasons: (1) to increase phylogenetic signal; and (2) as a modest check for the inadvertent PCR amplification of a nuclear pseudogene (Bermingham et al., 1996; Zhang and Hewitt, 1996). Concordant mtDNA gene phylogenies representing a minimum of 8000 bp of separation indicate either anticipated linkage across the mitochondrial molecule or intact translocation of a 8000 bp mitochondrial fragment to the nucleus (Lopez et al., 1994). The scnDNA Aldolase primer pair, ALD1 and ALD2 amplification product (Lessa and Applebaum, 1993), provided a gene region with slower substitution rates and an independent molecular assessment of *Scomberomorus* phylogeny.

Amplifications by the polymerase chain reaction (PCR; Saiki et al., 1985) were carried out in 50 μ l reactions: 5 μ l 10X buffer (100mM Tris pH 8.3, 20 mM MgCl₂, 500 mM KCL, 0.1% Gelatin), 4 μ l MgCl₂, 5 μ l 2mM dNTP, 0.25 μ l *Taq* polymerase, 29.75 μ l H₂O, 1 to 2 μ l genomic DNA as template, and the following primer

pairs (2.5 μ l of each 10mM primer): ATPase8.2 coupled to COIII.2 or COIII.3 for the ATPase genes, MET coupled to TRP or ASN for the ND2 genes (primer sequences available on request from EB), 12SA and 12SB (Kocher et al., 1989), and ALD1 and ALD2 (Lessa and Applebaum, 1993). All amplifications were conducted in a Perkin Elmer 480 or 9600 thermal cycler. ATPase and 12S amplifications involved 27 cycles of 94 C for 45 sec, 53 C for 45 sec, and 72 C for 90 sec. ND2 gene amplifications included 25 cycles with an annealing temperature of 52 C or 55 C depending on whether the template was genomic DNA or a reamplification. ALD gene products were initially amplified for 30 cycles under the following conditions: 94 C for 45 sec and combined annealing and extension at 60 C for 90 sec. ALD PCR products were reamplified for 25 cycles with annealing at 65 C for 45 sec, and extension at 72 C for 90 sec. Template DNAs for reamplifications were prepared by electrophoresing 5 μ l of the initial PCR product in a 2% TBE low melting point (LMP) agarose gel, taking pipet stabs from the ethidium bromide (EtBr) visualized amplification product, and resuspending the product in 30 μ l of sterile distilled water. Final amplification prod-

ucts (PCR) were electrophoresed in a 2% TAE (0.04 M Tris-acetate, 0.001M EDTA, pH 8.0; Maniatis et al., 1982) NuSieve® GTG® LMP agarose gel and stained with EtBr. The visualized band (usually one) was cut out and dissolved in 400 µl GeneClean® Spin Glassmilk at 56 C for 5 min and gel purified to remove incomplete amplification products and unincorporated d-NTPs, according to vendor's protocol (Bio101, Inc.). Sequencing reactions were carried out with the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc. ABI, 1990) developed for use with the ABI model 373A DNA Sequencing System. Cycle sequencing was carried out on a Perkin Elmer Geneamp 9600 as recommended by ABI and according to their protocol.

Sequence data were aligned, edited, and manipulated with Macvector sequence analysis software (vers. 4.5.2, Eastman Kodak Co., 1994) and *SeqEd* (vers. 1.00A, Applied Biosystems, ABI, 1990). Nucleotide composition and bias were examined using Sequencer (vers. 3.1, Kessing, pers. comm.) and Molecular Evolutionary Genetics Analysis (MEGA vers. 1.0; S. Kumar, K. Tamura, and M. Nei, 1993, unpubl.). Homogeneity of nucleotide frequencies across taxa was tested independently for each gene region (chi-square, $P > 0$, alpha 0.05) in Phylogenetic Analysis Using Parsimony (PAUP* vers. 4.0.0d64, prerelease; D. L. Swofford, Sinauer Assoc., Sunderland, MA, 1997, unpubl.). Nucleotide distances were calculated in MEGA and PAUP* using Kimura's 2-parameter method (K2p; Kimura, 1980) and the LogDet paralinear distance (Steel, 1994), and reported as genetic distance or percent sequence divergence. We presented and discussed K2p distances (Table 2) owing to reduced variance terms compared to more complex substitution models and to permit ease of comparison between other studies of vertebrate molecular systematics and biogeography, specifically those of geminate taxa (Knowlton et al., 1993; Bermingham et al. 1997). Neighbor-joining trees (Saitou and Nei, 1987) were computed independently for, ATPase 8, ATPase 6, ND2, the three mitochondrial gene regions combined, transversions alone, and ALD, using METREE (Rzhetsky and Nei, 1994). Neighbor-joining (NJ) and minimum evolution (ME) trees were examined using the gamma distribution shape parameter, alpha, with the branch lengths statistically tested for significance ($P > 0$) and the standard errors of the estimates calculated. The Partition-Homogeneity test (D. L. Swofford, Sinauer Assoc., Sunderland, MA, 1997, unpubl.) was applied to 100 replications of a heuristic search with character partitions set gene-

TABLE 2. KIMURA 2-PARAMETER DISTANCES FOR COMBINED MITOCHONDRIAL ATPASE 8, 6 AND ND2 GENE REGIONS ABOVE THE DIAGONAL AND ALDOLASE BELOW THE DIAGONAL. Only individuals with full mtDNA and aldolase data are included.

OTU	species	ID #	1	2	3	4	5	6	7	8	9	10	11	12	13
1	<i>maculatus</i>	HB265		0.0027	0.0027	0.0016	0.0021	0.0288	0.0288	0.0687	0.0587	0.0610	0.1045	0.1039	0.1806
2	<i>maculatus</i>	HB264	0.0000		0.0053	0.0021	0.0027	0.0305	0.0316	0.0692	0.0616	0.0628	0.1064	0.1057	0.1820
3	<i>maculatus</i>	HB261	0.0000	0.0000		0.0125	0.0125	0.0305	0.0305	0.0693	0.0592	0.0616	0.1058	0.1051	0.1820
4	<i>regalis</i>	STRI3836	0.0125	0.0125	0.0043		0.0005	0.0294	0.0305	0.0693	0.0604	0.0616	0.1058	0.1052	0.1813
5	<i>regalis</i>	HB872	0.0125	0.0125	0.0048	0.0000		0.0299	0.0311	0.0699	0.0610	0.0622	0.1064	0.1058	0.1820
6	<i>brasiliensis</i>	STRI4277	0.0125	0.0125	0.0125	0.0050	0.0050		0.0064	0.0640	0.0629	0.0641	0.1072	0.1052	0.1850
7	<i>brasiliensis</i>	HB704	0.0125	0.0125	0.0125	0.0050	0.0050	0.0000		0.0640	0.0629	0.0653	0.1039	0.1020	0.1821
8	<i>concolor</i>	HB966	0.0125	0.0125	0.0125	0.0049	0.0049	0.0049	0.0049		0.0476	0.0487	0.1073	0.1054	0.1953
9	<i>sierra</i>	HB857	0.0125	0.0125	0.0125	0.0049	0.0049	0.0049	0.0049	0.0000		0.0032	0.1055	0.1036	0.1918
10	<i>sierra</i>	HB1191	0.0125	0.0125	0.0125	0.0049	0.0049	0.0049	0.0049	0.0000	0.0000		0.1074	0.1055	0.1913
11	<i>tritor</i>	STRI4017	0.0261	0.0261	0.0261	0.0286	0.0286	0.0260	0.0260	0.0287	0.0288	0.0287		0.0037	0.1805
12	<i>tritor</i>	HB968	0.0261	0.0261	0.0261	0.0286	0.0286	0.0260	0.0260	0.0287	0.0288	0.0287	0.0000		0.1827
13	<i>guttatus</i>	STRI4720	0.0421	0.0421	0.0421	0.0358	0.0358	0.0358	0.0358	0.0388	0.0388	0.0389	0.0430	0.0430	

by-gene (ATPase 8, ATPase 6, and ND2) to determine the statistical validity of combining the mtDNA genes for phylogenetic analysis and genetic distance estimates. We used PAUP* distance/likelihood options to iteratively generate both the transition:transversion (Ts:Tv) ratio and gamma shape parameter, alpha. Maximum likelihood trees for each gene were estimated heuristically using the parameters calculated by PAUP*.

Cladistic analyses of both nucleotide and translated amino acid sequences for each mitochondrial protein coding region were carried out with PAUP* and MacClade (vers. 3.05, Maddison and Maddison, 1992, unpubl.). Parsimony trees were obtained with the branch-and-bound search strategy using three weighting strategies: (1) unweighted; (2) Farris successive weighting (Farris, 1969), where character values were scaled by the consistency index (obtained from an initial unweighted search) and iteratively adjusted through subsequent searches, until their values stabilized; and (3) 4:9:1 for first, second, and third codon positions, respectively, and empirically estimated Ts:Tv ratios for each gene region. Aldolase sequences were recoded with five possible states (0–4) to include both nucleotides and indels as character states (Appendices 2–3). Each multiple base pair indel was treated as a single character, reflecting unique mutational events. Our rationale reflects the fact that, if each base pair (bp) within a multiple bp indel was treated as an individual character, the longer indels (i.e., 3 vs 52 bp indel) would be disproportionately weighted. Aldolase sequence from other scombrids (*S. cavalla*, *S. commerson*, *S. guttatus*, *S. koreanus*, *S. plurilineatus*, *S. semifasciatus*, and the monotypic sister to *Scomberomorus*, *Acanthocybium solandri*) was used to confirm synapomorphic and sympleisiomorphic conditions and to confirm the monophyly of the *S. regalis* species group (results not shown). Finally, a combined character matrix was composed including scnDNA Aldolase characters, coded mitochondrial sequence data and the morphological data of Collette and Russo (1985a, 1985b) with parsimony cladograms generated using a branch and bound search (PAUP*). The disproportionate number of mitochondrial characters necessitated a cautious approach to total evidence analysis. Therefore, we used two methods to reduce the influence of the mtDNA data, thus more closely approximating the number of characters provided by the morphological and scnDNA datasets. In one approach, nodes in the mtDNA branch-and-bound topology were characters (a shared node is considered a synapomorphy), and in the second we used only mtDNA transversions.

RESULTS

The complete nucleotide sequences of the mitochondrial ATPase 6 (684 bp), ATPase 8 (168 bp), and ND2 (1047 bp) genes and 356 to 410 bp of aldolase intron 5 (and a small part of the flanking exon 5) were determined for multiple individuals representing the six species in the *S. regalis* group (Collette and Russo, 1985a) and one outgroup species, *S. guttatus* (Table 1). A second outgroup taxon, *S. cavalla*, was used to confirm the monophyly of the *S. regalis* group using the complete ATPase 6 and 8 genes, partial ND2 gene, and aldolase intron 5 (Table 1). We sequenced an additional eight individuals representing *S. brasiliensis* ($n = 5$), *S. maculatus* ($n = 2$), and *S. sierra* ($n = 1$) for ATPase 6 and 8. Additionally, approximately 400 bp of the 12S ribosomal mtDNA gene was sequenced in *S. maculatus* and *S. regalis* to increase the overall linkage distance between the mtDNA genes examined in these species (Table 1). This gene region was not included in the phylogenetic analyses.

Molecular characterization of Scomberomorus mtDNA.—All sequences were translated and compared to the published nucleotide and amino acid sequences of the ND2 and ATPase 6 and 8 genes of *Cyprinus carpio* (Huang, 1994, unpubl.; Genbank X61010). No termination codons were observed, and all sequences begin with an ATG start codon and end with TAA providing modest evidence that we have sequenced functional mtDNA genes. Chi-square tests indicated homogeneity of nucleotide frequencies across taxa ($P = 1.000$).

Nucleotide composition was consistent across the three mtDNA genes with the following mean percentages: 27.9% for adenine (A), 26.3% for thymine (T), 32.2% for cytosine (C), and 13.7% for guanine (G). Compositional differences across mtDNA genes were greatest for thymine, 23.5% in ATPase 8 and 28.4% in ATPase 6. However, nucleotide composition varied considerably across codon positions. For example within ND2, adenine contributed 31.5% of the nucleotides at the first codon position, 16.8% at the second and 37.7% at the third. Whereas in ATPase 6, adenine occurred at 14.9%, 39.5%, and 26.3% of the first, second, and third codon positions, respectively.

Rates of transition substitutions were significantly greater than transversion substitution rates. Ts:Tv ratios were 5.66 for ATPase 8, 6.40

for ATPase 6 and 5.70 for ND2, with a combined Ts:Tv ratio of 6.03. Transition:transversion ratios declined for comparisons among the most divergent taxa. Among-site rate variation was evident in the maximum likelihood estimates of the gamma parameter ($\alpha = 0.395$, ATPase 8; 0.138, ATPase 6; 0.167, ND2; 0.160 across the combined mtDNA genes), suggesting that most sites have very low substitution rates, whereas a few sites experience very high rates. First codon position substitutions accounted for 15.7% of the total, whereas 5.6% and 78.7% occurred at second and third positions, respectively.

Molecular characterization of *Scomberomorus Aldolase*.—PCR amplification with the ALD1 and ALD2 (Lessa and Applebaum, 1993) primer pair consistently yielded two bands, one approximately 400 bp in length and the second 200–300 bp in length. Initially, both bands were sequenced and compared to published sequences of aldolase intron 5 and flanking exons 5 and 6 (Slade et al., 1993; Lessa and Applebaum, 1993; Llewellyn et al., 1995), identifying the 400-bp aldolase product as aldolase exon 5/intron 5. Appendix 4 provides an alignment of cloned ALD B in sea bream, *Sparus aurata* (Llewellyn et al., 1995, Genbank accession number X82278), and the ALD alleles observed in the six members of the *S. regalis* species group and *S. guttatus*. Our ALD sequence begins at position 473 of *S. aurata* ALD-B locus. The first 110 bp of our sequence represent exon 5, and intron 5 begins at position 111. Due to indel variation in ALD, we recovered 356 to 410 bp across species of *Scomberomorus* species (Table 1). The smaller PCR fragment may be an aldolase pseudogene and was not studied. Nucleotide composition of ALD was similar to the mtDNA genes, with the following frequencies: 26.0% A, 25.3% T, 30.9% C, and 17.8% G. The Ts:Tv ratio in the ALD sequences was 1.33, and $\alpha = 0.006$.

Relationships within the *Scomberomorus regalis* species group.— We present minimum evolution (Figs. 3A, 4A) and maximum parsimony (Figs. 3B, 4B) trees. These trees represent the six species in the *S. regalis* species group and *S. guttatus*. Excepting the placement of *S. maculatus* in the mtDNA and aldolase trees, different methods of analysis including neighbor joining (NJ) using K2p distances, minimum evolution, maximum likelihood, and maximum parsimony yield the same or very similar hypotheses of relationship. Furthermore, the monophyly of the *S. regalis* species group was confirmed through

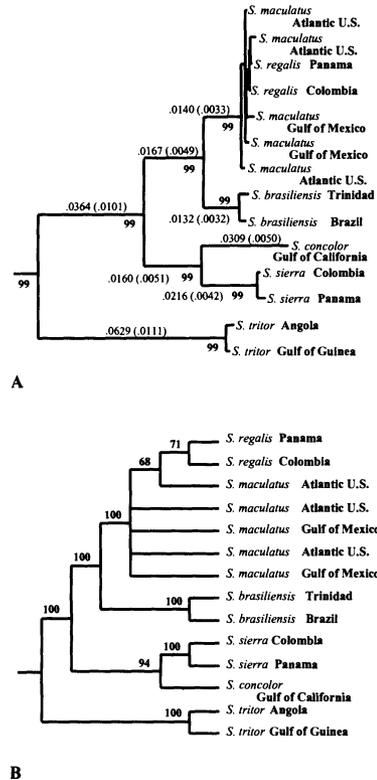


Fig. 3. (A) Minimum Evolution phenogram for the combined ATPase 8, ATPase 6, and ND2 *Scomberomorus* mitochondrial genes. Branch lengths (with standard errors of the estimates in parentheses) are shown above the branches; the probability that branch lengths are greater than zero are provided below in bold. (B) Strict consensus of 30 most-parsimonious trees based on a branch-and-bound search of the combined mtDNA data with bootstrap values based on 500 replicates. Tree Length (TL): 614 steps; Consistency Index (CI): 0.84; Homoplasy Index (HI): 0.17; Retention Index (RI): 0.84. Trees were rooted with the *S. guttatus* and *S. cavalla* outgroups (not shown).

unpublished analysis of additional outgroup taxa (*S. cavalla*, using mtDNA and ALD data; *S. commerson*, *S. guttatus*, *S. koreanus*, *S. plurilineatus*, *S. semifasciatus*, and *Acanthocybium solandri* for ALD).

The partition homogeneity test detected no significant differences between the three mtDNA gene partitions ($P = 0.880$). Thus, phylogenetic analyses were conducted on the combined mtDNA dataset. It is worth noting, however, that in all cases gene-by-gene analyses of the data are consistent with those obtained from the combined dataset. We observed 468 variable sites of which 271 are phylogenetically informative. Analyses of mtDNA indicate a close relationship of *S. maculatus* and *S. regalis* (Fig.

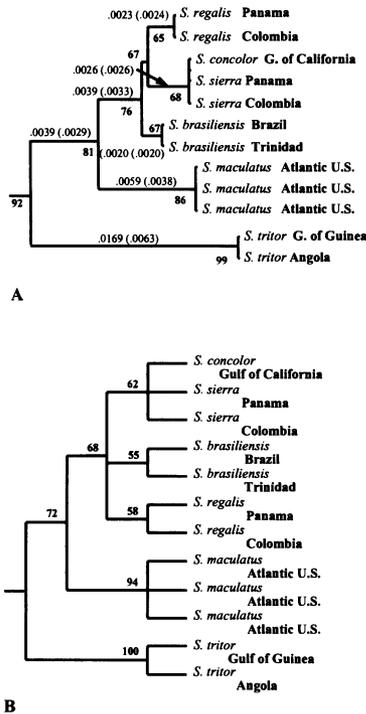


Fig. 4. (A) Minimum Evolution phenogram for *Scomberomorus* aldolase exon5/intron5 (356–408 bp). Branch lengths follow the convention presented in Figure 3. (B) The single most-parsimonious tree resulting from a branch-and-bound search of aldolase sequence data, recoded to include both nucleotides and indels as characters. Bootstrap values (in bold) are based on 500 replicates; TL: 33 steps; CI: 1.0; HI: 0.0; RI: 1.0. Trees were rooted with the *S. guttatus* and *S. cavalla* outgroups (not shown).

3). These species are indistinguishable on the basis of their mtDNA sequences. The lack of mtDNA divergence between *S. maculatus* and *S. regalis* mtDNAs was confirmed by sequencing additional individuals for ATPase 6,8 and partial 12S (Table 1). Across the geographic range represented by the two species, mtDNA distances were low; values ranged from 0.0005–0.0059 (mean 0.0030), resulting from a total of 10 mtDNA nucleotide substitutions among seven individuals (Table 2). The largest mtDNA genetic distance within the *regalis/maculatus* clade, 0.0059, was observed between a Gulf of Mexico and a Chesapeake Bay *S. maculatus*. Outside the *maculatus/regalis* mtDNA clade, the minimum mtDNA distance between mtDNA clades was 0.0288, observed between the *S. maculatus/regalis* and *S. brasiliensis* clades (Table 2).

Within the western Atlantic and eastern Pacific, intraocean species of *Scomberomorus* are more closely related than transisthmian species

(Fig. 3A). *Scomberomorus brasiliensis* was clearly distinguished from the western Atlantic species subsumed in the *S. maculatus/regalis* mtDNA clade, with distances ranging from 0.0288–0.0322 (mean 0.0304). The eastern Pacific species, *S. concolor* and *S. sierra* were separated by distances of 0.0476–0.0487 (mean 0.0482). Inter-ocean comparisons between eastern Pacific and western Atlantic species ranged from 0.0587–0.0711 (mean 0.0641).

A branch-and-bound search of the combined mtDNA nucleotide sequence data with the three weighting schemes described above, resulted in 30 most-parsimonious (MP) trees. Species relationships were congruent in all trees; only the phylogenetic order of mtDNA lineages within the combined *regalis/maculatus* clade differed. The three weighting schemes did result in somewhat different tree lengths and slightly different tree indices.

We recovered 31 polymorphic sites in aldolase intron sequences (Appendix 1). Indels represented a significant proportion of the scnDNA phylogenetic signal (Appendix 2). Nuclear-encoded ALD sequence strongly supported the distinctiveness of *S. maculatus* and *S. regalis* (genetic distance = 0.0127) and the basal position of *S. maculatus* within the New World taxa (Fig. 4).

In general, ALD genetic distances were considerably lower than mtDNA-based estimates. For example, the ALD genetic distance between the eastern Pacific species *S. concolor* and *S. sierra* was 0.000, compared to a mtDNA distance of 0.0482. The ALD genetic distance between western Atlantic *S. brasiliensis* and *S. sierra* was 0.0050, compared to a mtDNA distance of 0.0640. The genetic distance between *S. brasiliensis* and the eastern Atlantic *S. tritor* was 0.0260 for ALD and 0.1100 for mtDNA. Although the sample of nuclear-encoded substitutions is very small, there appears to be a five- to 10-fold difference in substitution rate between the aldolase intron and the mtDNA protein-coding genes. The variance in ALD distance estimates, particularly among closely related taxa, is high.

Combined morphological and molecular data.—The combined data analyses were based on single representatives of the seven species of *Scomberomorus* presented in Appendix 3 since no structured intraspecific variation was found. We faced two methodological quandaries in combining the three datasets for phylogenetic analysis. The first resulted from the different number of distinct *Scomberomorus* OTUs identified by mtDNA ($n = 6$), ALD ($n = 7$) and morphology

($n = 7$). Our response was to treat the mtDNA data for *S. maculatus* as missing. This assumption posits the loss of the *S. maculatus*, rather than the *S. regalis* mtDNA genome, owing to the derived position of the *maculatus/regalis* mtDNA and the derived position of *S. regalis* as compared to *S. maculatus* in the independent phylogenetic hypotheses for the *S. regalis* species group based on morphology and ALD.

Our second quandary owed to the vast differences in the number of phylogenetically informative characters provided by the three datasets; thus we sought to downweight the contribution of the mtDNA data. In combining the morphology and molecular characters, the mtDNA data were handled in three ways. In each treatment, the *S. maculatus* mtDNA was treated as missing. In the first approach, we utilized the total mtDNA evidence resulting in the single MP tree pictured in Figure 5A. This is the mtDNA tree (Fig. 3) except that *S. maculatus* was separated from *S. regalis* by the addition of the ALD and morphological data. Our second approach used only transversion substitutions to represent the mtDNA data. This analysis resulted in two MP trees, one reflecting the morphological topology (Fig. 2) and the other as pictured in Figure 5A. The consensus of the two MP trees is presented in Figure 5B. The third approach to the combined analysis of the three datasets used mtDNA characters generated from the mtDNA most-parsimonious tree; nodes = characters, shared nodes = synapomorphies, distilling 271 polymorphic characters to 6 characters (see Appendix 3). The analysis of this condensed dataset resulted in the single MP tree pictured in Figure 5A.

DISCUSSION

We employed an array of characters, both morphological and molecular, to elucidate the systematic relationships within the *S. regalis* species group. Our use of these three classes of character data illustrates the strength of a combined approach for inferring relationships within one *Scomberomorus* species group. Furthermore, a multileveled molecular approach permitted us to examine both recent divergences of closely related species and those occurring at deeper nodes suggesting more ancient separations. Our data provide an initial phylogenetic assessment of the diversification model(s) implied by Rosen's EP/EA biogeographic track.

The addition and analysis of molecular data confirmed the monophyly of the *S. regalis* species group and the basal position of *S. tritor*, presented in the previous morphology-based *Scom-*

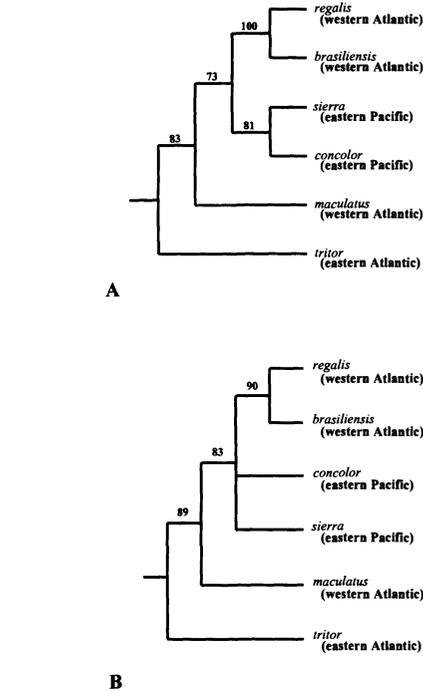


Fig. 5. Most-parsimonious trees (MP) resulting from the combined analysis of *Scomberomorus* morphology, mitochondrial DNA (1889 bp) and aldolase exon5/intron5 DNA (356–408 bp). Phylogenetic analyses varied with regard to the coding of the mitochondrial data but not the morphological and aldolase characters used (see text for details). (A) The single MP tree resulting from a branch-and-bound search of the combined data, using all mtDNA characters (TL: 631 steps; CI: 0.87; HI: 0.13; RI: 0.58) or recoded mtDNA characters (TL: 54 steps; CI: 0.94; HI: 0.06; RI: 0.79). (B) Strict consensus of the two MP trees resulting when only mtDNA transversion characters were used (TL: 235 steps; CI: 0.93; HI: 0.07; RI: 0.61).

beromorus hypothesis of Collette and Russo (1985a, 1985b). The new data, however, also indicated points of conflict internally and with the morphology-based phylogeny. The principal conflict involved *S. maculatus* and *S. regalis*, which share virtually identical mtDNA sequences but are otherwise easily distinguished on the basis of either aldolase sequence or morphology (discussed below). A second conflict regards the phylogenetic placement of the Gulf of California endemic, *S. concolor*. The mtDNA and scnDNA data strongly support a sister group relationship between the two eastern Pacific species, *S. concolor* and *S. sierra* (Figs. 3–4), whereas the morphological hypothesis places *S. concolor* as sister to a clade including *S. sierra* and the two derived western Atlantic species (Fig. 2). When the molecular data are constrained to fit

the morphological hypothesis, the resulting tree is 12 steps longer (with reduced consistency indices) than the shortest molecular tree. This outcome promotes the fully resolved *S. regalis* hypothesis presented in Figure 5A. In fact, confident placement of *S. concolor* with the data at hand, requires choosing between a complex morphological character and a single linked locus represented by multiple substitutions. Without additional data representing evolutionarily independent characters, we are unable to objectively determine which character provides the stronger phylogenetic signal. The consensus tree presented in Figure 5B, reflects the uncertain placement of *S. concolor*, while firmly establishing the derived sister relationship of *S. brasiliensis* and *S. regalis*, the transisthmian relationship of this western Atlantic clade to the eastern Pacific *S. sierra* and the basal position of *S. maculatus* among the New World members of the *S. regalis* species group.

Morphology and the aldolase data congruently identified *S. maculatus* as the basal member of the New World *S. regalis* clade (Figs. 2, 4, summarized in Fig. 5). In contrast, *S. maculatus* and *S. regalis* shared virtually identical mtDNA genomes (Fig. 3). These two well-differentiated species are distinguished by several aldolase characters (Appendix 3) and the following apomorphic morphological characters present in *S. regalis* and absent in *S. maculatus*: (1) elongate posterior pelvic process; (2) fourth right epi-branchial artery giving rise to a coeliaco-mesenteric shunt; and (3) loss of the pterotic spine (Collette and Russo, 1985a, 1985b). The *S. maculatus/regalis* mtDNA genome was most closely related to *S. brasiliensis*, one of the most derived *Scomberomorus* species. One explanation that we have considered for the *S. maculatus* and *S. regalis* mtDNA similarity was that our mtDNA results were compromised by the inclusion of a nuclear pseudogene. To address this concern, we undertook additional mtDNA sequencing (Table 1). The resulting congruence across the ATPase, ND2, and 12S ribosomal genes indicated that a mtDNA pseudogene was unlikely. An alternative, phylogenetically based interpretation would require the intact translocation of a 8000-bp mtDNA fragment to the nucleus (Lopez et al., 1994).

Under the assumption that we have sequenced functional mtDNA genes for both species, it is probable that the mtDNA similarity of *S. maculatus* and *S. regalis* resulted from hybridization. The extreme genetic similarity of the two mtDNA genomes suggests that hybridization between the species must have been very recent, or has been maintained to the present

(Table 2, Fig. 3A). However, sequence differences between nuclear-encoded aldolase alleles for *S. maculatus* and *S. regalis* would suggest that hybridization was ephemeral and is not ongoing. The morphology and aldolase data, coupled with the derived phylogenetic placement of the shared mtDNA, suggest the introgressive replacement of the *S. maculatus* mtDNA genome by that of *S. regalis*. Perhaps strong directional selection has acted to fix the *S. regalis* mtDNA type in *S. maculatus*. An alternative scenario would favor the stochastic loss of the *S. maculatus* mtDNA genome from a hybrid population carrying both *S. maculatus* and *S. regalis* mtDNA types against a predominately *S. maculatus* nuclear background. Small *S. maculatus* population size coupled to occasional spawnings between *S. maculatus* males and *S. regalis* females might promote such a scenario.

Scomberomorus maculatus and *S. regalis* are known to be seasonally sympatric in portions of their ranges, specifically in Yucatan, the northwestern Gulf of Mexico, southern Florida and periodically along the Atlantic coast as far north as Massachusetts (Collette and Nauen, 1983). The Campeche and Yucatan region of the Gulf of Mexico is a contact zone between the subtropical to temperate fauna of the Gulf of Mexico and the tropical Central American and Caribbean faunas (Briggs, 1974, and citations therein). Hemiramphidae of possible hybrid origin have been collected in this region (Banford and Collette, 1993). More specifically, ripe *S. maculatus* females are found from April through September in the waters around south Florida (Klima, 1959, unpubl.; Powell, 1975, unpubl.), and sexually mature *S. regalis* are present from August through October (Fincune and Collins, 1984). Thus, there are areas and periods of time where and when hybridization could occur between the two species.

Our experimental design did not anticipate the *S. maculatus/S. regalis* mtDNA similarity; thus a full evaluation of hybridization and introgression must await a more extensive geographic and molecular appraisal of these species. It is interesting to note that hybridization and mtDNA introgression against an apparently nonhybrid nuclear background has been suggested for *Thunnus*, another scombrid genus (Chow and Kishino, 1995).

Scomberomorus biogeography.—Molecular approaches to historical biogeography provide data that can be used to interpret both spatial and temporal patterns of relationship (Bermingham and Avise, 1986; Bermingham et al., 1992). In the case of the EP/EA biogeographic

model, the celebrated features are the Cretaceous opening of Atlantic Ocean and the Pliocene completion of the Central American Isthmus. Having established the geographic distribution (Fig. 1) and phylogeny (Fig. 5) for the *S. regalis* species group, we turn to a chronological assessment of diversification using independent molecular markers to determine whether the relative times since speciation are congruent (e.g., Vawter and Brown, 1986; Bermingham and Lessios, 1993). The putative loss of the *S. maculatus* mtDNA genome and the very limited nucleotide sampling of aldolase interferes with comparisons among some of the closely related members of the New World *Scomberomorus* clade. Nonetheless, the rank-order consistency of the independent mtDNA and aldolase genetic distances suggest that *Scomberomorus* lineages have not experienced grossly different rates of evolution. The ratio of mitochondrial to aldolase distance did vary across comparisons; whether this owed to modest differences in evolutionary rates or inadequate aldolase nucleotide sampling cannot be determined from our data. Generally, we observed a five- to 10-fold higher substitution rate in mtDNA protein coding genes as compared to aldolase exon 5/intron 5.

Congruent measures of molecular divergence indicated that we could proceed with some confidence to roughly date speciation events in the *S. regalis* species group. Our estimates of dates are based on the single calibration point provided by the Pliocene rise of the Isthmus of Panama (Coates and Obando, 1996, and references therein). We have dated *Scomberomorus* speciation from two perspectives. First, we estimated ages using an "average" teleost calibration for mtDNA protein-coding genes provided by Bermingham et al. (1997). Second, we calibrated a *Scomberomorus*-specific clock for mtDNA and aldolase. To circumvent uncertainty raised by the *S. maculatus*/*S. regalis* hybridization and the differential placement of *S. concolor* in the mtDNA and morphology-based phylogenetic hypotheses, we based our calibration on the data for the western Atlantic *S. brasiliensis* and eastern Pacific *S. sierra*. These two species represent transisthmian sister lineages in the morphology and mtDNA trees, and we assumed that the range of their common ancestor was split by the rise of the Central American Isthmus. The first calibration was based on mtDNA K2P divergence calculated for transisthmian pairs representing eight genera of marine teleosts, and a date of 3 million years for completion of the Isthmian barrier (Table 1; B cluster in Bermingham et al., 1997). The simple, arithmetic average of the eight mtDNA divergence values was 1.5% se-

quence divergence per million years with a range of 1.0–1.8%. The *Scomberomorus*-specific calibration was 2.1% for the mtDNA protein-coding genes and 0.2% per million years for aldolase. If the different mtDNA calibrations resulted from different rates of molecular evolution, it would suggest a faster mtDNA evolutionary rate for *Scomberomorus*. Alternatively, the transisthmian members of the *S. regalis* species group separated roughly 4–5 Ma, prior to the closure of the Isthmian seaway.

Both monophyly of the *S. regalis* species group and the basal position of the eastern Atlantic *S. tritor* support the EP/EA hypothesis that the Cretaceous opening and subsequent widening of the South Atlantic Ocean led to the original separation of the eastern Atlantic and New World forms. But do temporal estimates based on the genetic distances observed between *S. tritor* and the New World members of the *S. regalis* species group match the chronological predictions of the EP/EA biogeographic model? The opening of the South Atlantic Ocean has been geologically well dated (Emery and Uchupi, 1984; Parrish, 1993; Pittman et al., 1993), establishing 65–95 Ma as the maximum time to divergence between eastern and western Atlantic marine taxa. Present distribution and behavior of *Scomberomorus* suggest that, for as long as the proto-South Atlantic Ocean remained shallow and narrow, populations of the common ancestor were not isolated from one another. It appears from our molecular data that the separation of eastern Atlantic and New World *S. regalis* clade members occurred closer to the end of the Tertiary than in the late Cretaceous.

Our molecular time estimates based on the mtDNA and aldolase data suggest that the divergence between *S. tritor* and the New World *S. regalis* group members is probably no older than mid-Miocene (5–7 Ma for mtDNA; 14 Ma for Aldolase). These dates suggest that the mid-Miocene closure of the Tethys Sea (Vrielynck et al., 1997) and the widening ocean basin (Scheltema, 1995; Feldman et al., 1998) may have caused the separation of eastern and western Atlantic *Scomberomorus* species. Scheltema (1995) presented a similar argument in regard to the distribution patterns of tropical Atlantic mollusks and indicated that the mid-Atlantic serves as a filter barrier that decreases in permeability through the late Tertiary. Furthermore, Caribbean fish fossils indicate a shift from a Tethyan to modern teleost fauna at the Oligocene–Miocene boundary (Nolf and Stringer, 1992; Nolf and Aguilera, 1998).

The New World members of the *S. regalis*

group are relatively young, and our aldolase data indicate a basal split at roughly 6.3 Ma. Although the split predates the Pliocene closure of the Central American Isthmus, the development of physical and oceanographic changes between the Caribbean and the eastern Pacific had already begun (Duque-Caro, 1990a, 1990b; Coates et al., 1992). These changes have been implicated in the divergence of New World tropical marine taxa (Knowlton et al., 1993; Lessios et al., 1995; Bermingham et al., 1997), but specific isolating mechanisms have not been elaborated. The geographic distribution (Fig. 1) and age of *S. maculatus* suggests that the low sea level stand at the close of the Miocene (Haq et al., 1987) and deflection of the Gulf Stream (Berggren and Hollister, 1974; Berggren, 1978) may have isolated this species in the Gulf of Mexico.

The relative phylogenetic position and genetic distances presented by the four derived members of the New World *S. regalis* clade varied. The sister-group relationships of the eastern Pacific *S. concolor* and *S. sierra*, and the western Atlantic *S. brasiliensis* and *S. regalis* inferred from mtDNA, provided evidence for two intraoceanic divergence events postdating the split between transisthmian lineages. Using the average teleost mtDNA calibration suggested that the completion of the Isthmian barrier triggered these intraoceanic speciation events, following an early Pliocene separation of transisthmian lineages (about 4.3 Ma). However, the shorter mtDNA branch lengths observed in the western Atlantic *Scomberomorus* pair indicated that these sister species may have originated after the separation of *S. concolor* and *S. sierra*. This distinction is further emphasized by use of the *Scomberomorus* specific mtDNA calibration suggesting that the western Atlantic species diverged at 1.4 Ma (average calibration yields 2 Ma), well after closure of the Isthmian seaway. Isolation and speciation within the tropical western Atlantic (Bowen and Avise, 1990; Reeb and Avise, 1990; Knowlton and Weigt, 1998) may have occurred during Pleistocene glaciations when sea levels were appreciably lower than present, restricting connections between basins (Poag, 1973). Within the eastern Pacific, we estimated speciation at 2.3 Ma (average calibration yields 3.1–3.2 Ma). Presently, *S. concolor* is restricted to the Gulf of California, an area of high endemism (Walker, 1960; Durham and Allison, 1960; Thomson and Gilligan, 1983). A combination of physical factors most likely played a role in eastern Pacific speciation. The Miocene/Pliocene (Gastil et al., 1983) formation of the Gulf of California, the historical occurrence of strong upwelling events

(Hubbs, 1960), and oceanographic changes resulting from closure of Isthmian seaways (Keigwin, 1982; Duque-Caro, 1990a, 1990b) are candidates for isolating barriers.

The *S. regalis* species group clearly illustrates a general feature of the EP/EA track, namely, the lower species diversity in the eastern Atlantic relative to the New World tropics (Briggs, 1974, and citations therein, 1985). Higher speciation rates in the western Atlantic region (especially tropical) may be attributed to its greater geologic and topographic complexity as compared to the eastern Atlantic. The Gulf of Mexico and Caribbean Sea, with associated island chains and backarc basins, presently and historically have had restricted circulation (Berggren, 1978; Broecker and Denton, 1990; Mooers and Maul, 1998). If one takes into account historical sea level change (Haq et al., 1987), the complexity of the western Atlantic region becomes more exaggerated. Conversely, the continental margin and coast of West Africa are topographically simple with minor relief and, thus, present little to impede movements of a coastal migrant other than water temperature bounding the tropical region. It is also possible that the greatly reduced shallow shelf area of the eastern Atlantic relative to the New World tropics (Ajao and Houghton, 1998) has led to higher extinction rates in the eastern Atlantic.

In sum, our use of multiple markers advanced a more robust inference of phylogenetic relationships for the *S. regalis* group than would have been obtained from any single character set. For example, neither morphology nor aldolase identified the putative history of hybridization between *S. maculatus* and *S. regalis*. Conversely, reliance on the mtDNA phylogeny would have led to an incorrect conclusion regarding the magnitude of differentiation and phylogenetic placement of *S. maculatus*. Furthermore, our use of the unlinked mitochondrial and aldolase genes provided independent estimates regarding the chronological divergence of *Scomberomorus* species. Our systematic study of the *S. regalis* species group permitted a positive geographical and chronological assessment of the diversification model(s) implied by EP/EA biogeographic track. Evaluation and refinement of the generalized EP/EA marine track will progress with temporally and spatially based analyses of phylogenetic hypotheses for additional taxa.

ACKNOWLEDGMENTS

This work was supported by Smithsonian Institution (SI) and Smithsonian Tropical Re-

search Institute (STRI) graduate fellowships, and National Marine Fisheries Service (NMFS) internships to HB, National Geographic Grant In-Aid of Field Research (5425–95) to BBC and HB, and the STRI Molecular Evolution Program. For fresh tissue samples, we thank G. Bianchi, L. Barbieri, S. Lowerre-Barbieri, C. Cooksey, S. Gaichas, C. Monteiro-Neto, and J. Caruso. Scientific collecting permits from Recursos Marinos and the Kuna Comarca, Panama, are gratefully acknowledged. Review of the manuscript and comments by A. Cooper, D. Scoles, and M. Vecchione was greatly appreciated. HB thanks J. Milliman, J. A. Musick, and the SMS/VIMS graduate program for assistance and stipend support while in residence at STRI. And a very special thanks goes to N. Gomez for her enthusiasm in getting HB started sequencing. This research is in partial fulfillment of HB's Ph.D. dissertation, SMS/VIMS, College of William and Mary. I especially wish to thank my wonderful mentor for once agreeing with me.

LITERATURE CITED

- AJAO, E. A., AND R. W. HOUGHTON. 1998. Coastal Ocean of equatorial West Africa from 10°N to 10°S, Coastal segment (17,E), p. 605–631. *In: The sea*. Vol. 11. A. R. Robinson and K. H. Brink (eds.). John Wiley and Sons, Inc., New York.
- BANFORD, H. M., AND B. B. COLLETTE. 1993. *Hyporhamphus meeki*, a new species of halfbeak (Teleostei: Hemiramphidae) from the Atlantic and Gulf Coasts of the United States. *Proc. Biol. Soc. Wash.* 106: 369–384.
- BERGGREN, W. A. 1978. Recent advances in Cenozoic planktonic foraminiferal biostratigraphy, biochronology, and biogeography: Atlantic Ocean. *Micro-paleontology* 24:337–370.
- , AND C. D. HOLLISTER. 1974. Paleogeography, paleobiogeography and history of circulation in the Atlantic Ocean, p. 126–186. *In: Studies in Paleooceanography*. W. W. Hay (ed.). Society Of Economic Paleontologists and Mineralogists, Spec. Publ. 20, Tulsa, OK.
- BERMINGHAM, E., AND J. AVISE. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics* 113:939–965.
- , AND H. LESSIOS. 1993. Rate variation of protein and mitochondrial DNA evolution as revealed by sea urchins separated by the Isthmus of Panama. *Proc. Natl. Acad. Sci. USA*. 90:2734–2738.
- , S. ROHWER, S. FREEMAN, AND C. WOOD. 1992. Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: a test of Mengel's model. *Ibid.* 89:6624–6628.
- , G. SEUTIN, AND R. E. RICKLEFS. 1996. Regional approaches to conservation biology: RFLPs, DNA sequences, and Caribbean birds, p. 104–124. *In: Molecular genetic approaches in conservation*. T. B. Smith and R. K. Wayne (eds.). Oxford Univ. Press, New York.
- , S. MCCAFFERTY, AND A. MARTIN. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus, p. 113–128. *In: Molecular systematics of fishes*. T. Kocher and C. Stepien (eds.). Academic Press, San Diego, CA.
- BOWEN, B. W., AND J. C. AVISE. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life-history patterns. *Mar. Biol.* 107:371–381.
- BRIGGS, J. C. 1974. *Marine zoogeography*. McGraw-Hill, New York.
- . 1985. Species richness among the tropical shelf. *Sov. J. Mar. Biol.* 11:295–302.
- BROECKER, W. S., AND G. H. DENTON. 1990. What drives glacial cycles. *Sci. Am.* 262:48–56.
- CHOW, S., AND H. KISHINO. 1995. Phylogenetic relationships between tuna species of the genus *Thunnus* (Scombridae: Teleostei): inconsistent implications from morphology, nuclear and mitochondrial genomes. *J. Mol. Evol.* 41:741–748.
- COATES, A. G., AND J. A. OBANDO. 1996. The Geologic Evolution of the Central American Isthmus, p. 21–56. *In: Evolution and environment in tropical America*. J. B. C. Jackson, A. F. Budd, and A. G. Coates (eds.). Univ. of Chicago Press, Chicago.
- , J. B. C. JACKSON, L. S. COLLINS, T. M. CRONIN, H. J. DOWSETT, L. M. BYBELL, P. JUNG, AND J. A. OBANDO. 1992. Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and Panama. *Geol. Soc. Am. Bull.* 104:814–828.
- COLLETTE, B. B., AND C. E. NAUEN. 1983. FAO species catalogue. Vol. 2. Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos, and related species known to date. FAO, Rome.
- , AND J. L. RUSSO. 1981. A revision of the scaly toadfishes, genus *Batrachoides*, with descriptions of two new species from the eastern Pacific. *Bull. Mar. Sci.* 3:197–233.
- , AND ———. 1985a. Interrelationships of the Spanish mackerels (Pisces: Scombridae: *Scomberomorus*) and their copepod parasites. *Cladistics* 1: 141–158.
- , AND ———. 1985b. Morphology, systematics, and biology of the Spanish mackerels (*Scomberomorus* Scombridae). *Fish. Bull. USA* 82:545–692.
- DUQUE-CARO, H. 1990a. Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South America and the evolution of the Panama Seaway. *Palaeogeog. Palaeoclim. Palaeoecol.* 77:203–234.
- . 1990b. The Choco Block in the northwestern corner of South America: structural, tectonostratigraphic, and paleogeographic implications. *J. S. Am. Earth Sci.* 3:71–84.
- DURHAM, J. W., AND E. C. ALLISON. 1960. The geologic history of Baja California and its marine faunas. Symposium: the biogeography of Baja California and adjacent seas. Part I. Geologic history. *Syst. Zool.* 9:47–91.

- EMERY, K. O., AND E. UCHUPI. 1984. The geology of the Atlantic Ocean. Springer-Verlag, New York.
- FARRIS, J. S. 1969. A successive approximations approach to character weighting. *Syst. Zool.* 18:374–385.
- FELDMAN, R. M., K. L. BICE, C. S. HOPKINS, E. W. SALVA, AND K. PICKFORD. 1998. Decapod Crustaceans from the Eocene Castle Hayne Limestone, North Carolina: paleoceanographic implications. *J. of Paleon.* (Suppl. 1) 72:1–28.
- FINUCANE, J. H., AND L. A. COLLINS. 1984. Reproductive biology of cero, *Scomberomorus regalis*, from the coastal waters of south Florida. *Northeast Gulf Sci.* 7:101–107.
- FUTAI, M., T. NOUMI, AND M. MAEDA. 1989. ATP SYNTHASE (H⁺-ATPase): results by combined biochemical and molecular biological approaches. *Annu. Rev. Biochem.* 58:111–136.
- GASTIL, G., J. MINCH, AND R. P. PHILLIPS. 1983. The geology and ages of the islands, p. 13–25. *In: Island biogeography in the Sea of Cortéz.* T. J. Case and M. L. Cody (eds.). Univ. of California Press, Berkeley.
- HAQ, B. U., J. HARDENBOL, AND P. R. VAIL. 1987. Chronology of fluctuating sea levels since the Triassic. *Science* 235:1156–1167.
- HELFMAN, G. S., B. B. COLLETTE, AND D. E. FACEY. 1997. The diversity of fishes. Blackwell Science Inc., Malden, MA.
- HUBBS, C. L. 1960. The marine vertebrates of the outer coast. Symposium: the biogeography of Baja California and adjacent seas. *Syst. Zool.* 9:134–147.
- JORDAN, D. S. 1908. The law of geminate species. *Am. Nat.* 42:73–80.
- KEIGWIN JR., L. B. 1982. Isotopic paleoceanography of the Caribbean and East Pacific: role of Panama uplift in Late Neogene time. *Science* 217:350–353.
- KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–120.
- KNOWLTON, N., AND L. A. WEIGT. 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proc. Biol. Soc. Lond.* 265:1413.
- , ———, L. A. SOLÓRZANO, D. K. MILLS, AND E. BIRMINGHAM. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* 260:1629–1632.
- KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS AND S. PÄÄBO. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* 86:6196–6200.
- LESSA, E., AND G. APPLEBAUM. 1993. Screening techniques for detecting variation in DNA sequences. *Mol. Ecol.* 2:121–129.
- LESSIOS, H., G. R. ALLEN, G. M. WELLINGTON, AND E. BIRMINGHAM. 1995. Genetic and morphological evidence that the eastern Pacific damselfish *Abudefduf declivifrons* is distinct from *A. concolor* (Pomacentridae). *Copeia* 1995:277–288.
- LEWELLYN, L., V. P. RAMSURN, G. E. SWEENEY, T. WIGHAM, C. R. SANTOS, AND D. M. POWER. 1995. Cloning and characterisation of a fish aldolase B gene. *Biochim. Biophys. Acta* 1263:75–78.
- LOPEZ, J. V., N. YUHKI, R. MASUDA, W. MODI, AND S. J. O'BRIEN. 1994. *Numt*, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. *J. Mol. Evol.* 39:174–190.
- MANIATIS, T., E. F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- METZELAAR, J. 1919. Report on the fishes collected by Dr. J. Boeke in the Dutch West Indies 1904–1905: with comparative notes on marine fishes of tropical West Africa. Amsterdam, The Netherlands.
- MOOERS, C. N. K., AND G. A. MAUL. 1998. Intra-Americas Sea circulation, coastal segment (3,W), p. 183–207. *In: The sea.* Vol. 11. A. R. Robinson and K. H. Brink (eds.). John Wiley and Sons, Inc., New York.
- NAGLEY, P. 1988. Eukaryote membrane genetics: the F₀ sector of mitochondrial ATP synthase. *TIG* 4:46–52.
- NOLF, D., AND O. AGUILERA. 1998. Fish otoliths from the Cantaure Formation (Early Miocene of Venezuela). *Bull. de L'Institut Royal des Sciences Naturelles de Belgique. Sciences de la Terre* 68:237–262.
- , AND G. L. STRINGER. 1992. Neogene paleontology in the northern Dominican Republic 14. Otoliths of teleostean fishes. *Bull. Am. Paleontol.* 102:45–73.
- PARRISH, J. T. 1993. The Palaeogeography of the opening South Atlantic, p. 8–27. *In: The Africa-South America connection.* W. George and R. Lavocat (eds.). Clarendon Press, Oxford.
- PITTMAN, W. C. I., S. CANDE, J. LABRECQUE, AND J. PINDELL. 1993. Fragmentation of Gondwana: the separation of Africa from South America, p. 15–34. *In: Biological relationships between Africa and South America.* P. Goldblatt (ed.). Yale Univ. Press, New Haven, CT.
- POAG, C. W. 1973. Late Quaternary sea levels in the Gulf of Mexico. *Gulf Coast Assoc. Geol. Socs. Trans.* 23:394–400.
- RAGAN, C. I. 1987. Structure of NADH-Ubiquinone Reductase (Complex I). *Curr. Topics Biogenergetics* 15:1–36.
- REEB, C. A., AND J. C. AVISE. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. *Genetics*, Austin, Tex. 124:397–406.
- ROSEN, D. E. 1975. A vicariance model of Caribbean biogeography. *Syst. Zool.* 24:431–464.
- RZHETSKY, A., AND M. NEI. 1994. METREE: a program package for inferring and testing minimum-evolution trees. *CABIOS* 10:409–412.
- SAGHAI-MAROOF, M. A., K. M. SOLIMAN, R. A. JORGENSEN, AND R. A. ALLARD. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA* 81:8014–8019.
- SAIKI, R. K., S. SCHARF, F. FALOONA, K. B. MULLIS, G. T. HORN, H. A. ERLICH, AND N. ARNHEIM. 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–1354.

- SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for constructing phylogenetic trees. *Mol. Biol. Evol.* 4:406–425.
- SHELTEMA, R. S. 1995. The relevance of passive dispersal for biogeography of Caribbean mollusks. *Am. Malacol. Bull.* 11:99–115.
- SEUTIN, G., B. N. WHITE, AND P. T. BOAG. 1990. Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* 69:82–90.
- SLADE, R. W., C. MORITZ, A. HEIDEMAN, AND P. T. HALE. 1993. Rapid assessment of single-copy nuclear DNA variation in diverse species. *Mol. Ecol.* 2: 359–373.
- STEEL, M. A. 1994. Recovering a tree from the Markov leaf colourations it generates under a Markov model. *Appl. Math. Lett.* 7:19–23.
- THOMSON, D., AND M. GILLIGAN. 1983. The rocky-shore fishes, p. 98–129. *In: Island biogeography in the Sea of Cortéz.* T. J. Case and M. L. Cody (eds.) Univ. of California Press, Berkeley.
- VAWTER, L., AND W. M. BROWN. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. *Science* 234:194–196.
- , R. ROSENBLATT, AND G. C. GORMAN. 1980. Genetic divergence among fishes of the eastern Pacific and the Caribbean: support for the molecular clock. *Evolution* 34:705–711.
- VRIELYNCK, B., G. S. ODIN, AND J. DERCOURT. 1997. Miocene palaeogeography of the Tethys Ocean; potential global correlations in the Mediterranean, p. 157–165. *In: Miocene stratigraphy: an integrated approach.* A. Montanari, G. S. Odin, and R. Coccioni (eds.). Elsevier Science, Amsterdam, The Netherlands.
- WALKER, B. W. 1960. The distribution and affinities of the marine fish fauna of the Gulf of California. Symposium: the biogeography of Baja California and adjacent seas. *Syst. Zool.* 9:123–133.
- ZHANG, D., AND G. M. HEWITT. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. *Trends Ecol. Evol.* 11:247–251.
- (HMB, EB, SSM) SMITHSONIAN TROPICAL RESEARCH INSTITUTE, APARTADO 2072, BALBOA, REPUBLIC OF PANAMA OR SMITHSONIAN TROPICAL RESEARCH INSTITUTE, UNIT 0948, APO, AA 34002-0948; (HMB, BBC) VIRGINIA INSTITUTE OF MARINE SCIENCE, GLOUCESTER POINT, VIRGINIA 23062; AND (HMB, BBC) NATIONAL MARINE FISHERIES SERVICE, NATIONAL SYSTEMATICS LABORATORY, NATIONAL MUSEUM OF NATURAL HISTORY, WASHINGTON, DC 20560-0153. PRESENT ADDRESS: (SSM) 1214A MANGO DRIVE, YIGO, GUAM 96929. E-mail: (HMB) banfordh@naos.si.edu. Send reprint requests to HMB. Submitted: 24 July 1998. Accepted: 10 Feb. 1999. Section editor: J. D. McEachran.

APPENDIX 1.

MUSEUM CATALOG INFORMATION AND GENE BANK ACCESSION NUMBERS FOR *Scomberomorus* MATERIAL SEQUENCED.

- S. regalis*
 HB872 (USNM343341); ATPase8, 6 AF076103, ND2 AF076126, aldolase AF076147
 STRI3836 (USNM?); ATPase8, 6 AF076100, ND2 AF076124, aldolase AF076143
 STRI3973 (USNM343344); ATPase8, 6 AF076120
 STRI3977 (USNM343355); ATPase8, 6 AF076121
- S. brasiliensis*
 HB700; ATPase8, 6 AF076107
 HB701; ATPase8, 6 AF076106
 HB702; ATPase8, 6 AF076105
 HB704; ATPase8, 6 AF076108, ND2 AF076130, aldolase AF076145
 HB705; ATPase8, 6 AF076110
 STRI4276 (STRI-4276); ATPase8, 6 AF076111
 STRI4277 (STRI-4277); ATPase8, 6 AF076109, ND2 AF076129, aldolase AF076144
- S. sierra*
 HB857 (USNM343347); ATPase8, 6 AF076112, ND2 AF076132, aldolase AF076146
 HB948 (USNM?); ATPase8, 6 AF076114
 HB1191 (USNM?); ATPase8, 6 AF076113, ND2 AF076133, aldolase AF076139
- S. concolor*
 HB966 (USNM344347); ATPase8, 6 AF076115, ND2 AF076131, aldolase AF076148
- S. maculatus*
 HB260 (VIMS9587); ATPase8, 6 AF076097
 HB261 (VIMS9588); ATPase8, 6 AF076098, ND2 AF076127, aldolase AF076140
 HB264 (VIMS9591); ATPase8, 6 AF076104, ND2 AF076123, aldolase AF076141
 HB265 (VIMS9592); ATPase8, 6 AF076096, ND2 AF076122, aldolase AF076142
 HB266 (VIMS9593); ATPase8, 6 AF076099
 STRI5090; ATPase8, 6 AF076101, ND2 AF076125
 STRI5093; ATPase8, 6 AF076102, ND2 AF076128
- S. tritor*
 HB968 (USNM?); ATPase8, 6 AF076117, ND2 AF076135, aldolase AF076150
 STRI4017; ATPase8, 6 AF076116, ND2 AF076134, aldolase AF076149
- S. guttatus*
 STRI4720 (USNM?); ATPase8, 6 AF076118, ND2 AF076136, aldolase AF076151
- S. cavalla*
 HB803 (USNM?); ATPase8, 6 AF076119, ND2 AF076137, aldolase AF076138

APPENDIX 2. ALDOLASE CHARACTER STATE CHANGES ACROSS THE SEVEN *Scomberomorus* SPECIES ALIGNED. 0 = adenine, 1 = cytosine, 2 = guanine, 3 = thymine, 4 = indel; TS = transition, TV = transversion. Number of base pairs within an indel are in parentheses. Position refers to nucleotide position within the sequenced region.

Char. #	States	Description	Position
1	0, 2	TS	47
2	1, 3	TS	54
3	1, 3	TS	92
4	0, 4	indel (2)	118–119
5	0/3, 4	indel (5–12)	122–141
6	1, 3	TS	168
7	2, 4	indel (1)	183
8	2, 4	indel (1)	184
9	2, 4	indel (1)	186
10	3, 4	indel (1)	195
11	1, 3	TS	196
12	2, 3	TV	203
13	2, 4	indel (1)	205
14	0, 2	TS	213
15	0, 2	TS	216
16	1, 2	TV	281
17	0, 3	TV	289
18	1, 3	TS	292
19	0, 1, 2	TV; TS	296
20	2, 3	TV	305
21	0, 2	TS	306
22	2, 3	TV	316
23	0, 1	TV	323
24	1, 3	TS	325
25	0–3, 4	indel (55)	340–395
26	1, 2	TV	344
27	1, 3	TS	346
28	0, 2	TS	348
29	0, 1	TV	365
30	0, 3	TV	367
31	0, 1, 3	TV; TS	382

APPENDIX 3. DATA MATRIX FOR ALDOLASE, mtDNA AND MORPHOLOGICAL PARSIMONY ANALYSIS. Characters 1 through 31 represent aldolase (see Appendix 2 for definition of characters); characters 32 through 43 are morphological from Collette and Russo (1985) where state 5 = 1, 6 = 2, and 8 = 0; and character 32 = 6, 33 = 8, 34 = 9, 35 = 10, 36 = 11, 37 = 14, 38 = 15, 39 = 16, 40 = 17, 41 = 20, 42 = 2, 43 = 22; characters 44 through 49 are mtDNA nodes. * Missing data, all contained within indel character 25; ** treated as missing mtDNA genome, if treated as present it would be identical to *Scomberomorus regalis*, see text.

species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<i>tritor</i>	2	1	3	4	4	3	4	4	2	3	3	3	4	0	0	2	0	3	0	2	2	2	1	1	1
<i>concolor</i>	0	1	3	4	0	3	4	0	4	3	3	3	4	2	0	2	3	3	1	3	2	2	0	1	1
<i>sierra</i>	0	1	3	4	0	3	4	0	4	3	3	3	4	2	0	2	3	3	1	3	2	2	0	1	1
<i>brasiliensis</i>	0	1	3	4	0	3	4	0	4	3	3	3	4	2	0	2	3	3	1	2	2	2	0	1	1
<i>regalis</i>	0	1	3	4	0	3	4	0	4	3	3	3	4	2	0	2	3	3	1	2	2	2	0	1	1
<i>maculatus</i>	0	1	3	4	0	3	4	0	4	4	1	3	4	2	0	2	3	3	1	2	2	3	0	1	1
<i>guttatus</i>	2	3	1	0	0	1	2	0	4	3	3	2	2	2	2	1	3	1	2	2	0	2	0	3	4
species	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	
<i>tritor</i>	1	1	2	0	3	0	8	5	5	5	5	8	5	8	5	8	5	8	1	1	0	0	0	0	
<i>concolor</i>	2	1	0	1	0	1	8	5	5	5	5	8	8	5	5	8	5	5	1	1	1	1	0	0	
<i>sierra</i>	2	1	0	1	0	1	8	5	5	5	5	8	8	5	5	8	5	6	1	1	1	1	0	0	
<i>brasiliensis</i>	2	1	0	1	0	3	8	5	5	8	5	8	5	5	5	8	5	6	1	1	1	0	1	0	
<i>regalis</i>	2	3	0	1	0	1	8	5	5	5	5	8	5	5	5	8	5	6	1	1	1	0	1	1	
<i>maculatus</i>	1	1	2	1	0	1	8	5	5	5	5	8	8	8	5	8	5	5	**	**	**	**	**	**	
<i>guttatus</i>	*	*	*	*	*	*	5	8	8	8	6	5	5	8	8	5	8	8	1	0	0	0	0	0	

APPENDIX 4. ALDOLASE ALIGNMENT.

	50	100
Reference	ATTTCCCAAGTGGCTGTGTGTTAAAGATCACCTTACCACCCCTCC AACCTGGCCATCATGGGAACGCCAATGTGCTGGCCCGCTACGCCAGCAT	100
<i>S. maculatus</i>C.....C.....A.....T.....
<i>S. regalis</i>C.....C.....A.....T.....
<i>S. brasiliensis</i>C.....C.....A.....T.....
<i>S. sierra</i>C.....C.....A.....T.....
<i>S. concolor</i>C.....C.....A.....T.....
<i>S. tritor</i>C.....C.....T.....
<i>S. guttatus</i>C.....C.....T.....T.....
<i>Sparus aurata</i>A.G.....CC.C.....T.GGACGG.TG.....T.GCT..C.....C.T..A.A.....200
exon 5/intron 5	150	
Reference	CTGCCAGATG/GTTAGCCACACACACACACACTTAAACACACA CATTTACATTCACAGCTCTACAGCTCAGTGAAGAAGACCTATGTTTTCTG	
<i>S. maculatus</i>/.....-.....C.....T.....C--C.....>
<i>S. regalis</i>/.....-.....C.....T.....C--C.....>
<i>S. brasiliensis</i>/.....-.....C.....T.....C--C.....>
<i>S. sierra</i>/.....-.....C.....T.....C--C.....>
<i>S. concolor</i>/.....-.....C.....T.....C--C.....>
<i>S. tritor</i>/.....-.....C.....T.....C--C.....>
<i>S. guttatus</i>/.....-.....C.....C--C.....>
<i>Sparus aurata</i>/AACG...TGGTG.CT.TTGT...-.GC..GAG.TC.TGC.T >	300
Reference	250	
Reference	CATTGCAGTGATGTAACACAAACCACAGCTCTCAAACAGCTGTTACACA ACTCAGCACACTGTTGGTTCATCAGCTTCTCTTCTAACTAATTTCTGTACC	
<i>S. maculatus</i>-.....G.C.....>
<i>S. regalis</i>-.....G.C.....>
<i>S. brasiliensis</i>-.....G.C.....>
<i>S. sierra</i>	R...-.....G.C.....>
<i>S. concolor</i>-.....G.C.....>
<i>S. tritor</i>-.....A.....G.A.....>
<i>S. guttatus</i>G.....C.G.....>
	350	400

APPENDIX 4. CONTINUED

	350	400
Reference	AATTGGTACACAAAAGAGGAGAGCTATTGTCTGTGTTGTCATGACACTGGT	TGCTGTGCTCTCTACAAATCCAACTCTAAAACGCCCTCCCTGTGCTGTCCCCC
<i>S. maculatus</i>T.....G.....T.....
<i>S. regalis</i>GGT.A.....T.....
<i>S. brasiliensis</i>GG.A.....T.....
<i>S. sierra</i>T.....GG.A.....T.....
<i>S. concolor</i>T.....GG.A.....T.....
<i>S. tritor</i>G.....A.T.....A.....T.....
<i>S. guttatus</i>A.....T.....-----T.....
Reference	CCTCAGCATGGCATTGTGCCCA	
<i>S. maculatus</i>	> 407
<i>S. regalis</i>	> 408
<i>S. brasiliensis</i>	> 408
<i>S. sierra</i>	> 408
<i>S. concolor</i>	> 408
<i>S. tritor</i>	> 391
<i>S. guttatus</i>	..C.....	> 356

LINKED CITATIONS

- Page 1 of 2 -



You have printed the following article:

Phylogenetic Systematics of the *Scomberomorus regalis* (Teleostei: Scombridae) Species Group: Molecules, Morphology and Biogeography of Spanish Mackerels

Heidi M. Banford; Eldredge Bermingham; Bruce B. Collette; S. Shawn McCafferty

Copeia, Vol. 1999, No. 3. (Aug. 2, 1999), pp. 596-613.

Stable URL:

<http://links.jstor.org/sici?sici=0045-8511%2819990802%293%3A1999%3A3%3C596%3APSOTSR%3E2.0.CO%3B2-C>

This article references the following linked citations. If you are trying to access articles from an off-campus location, you may be required to first logon via your library web site to access JSTOR. Please visit your library's website or contact a librarian to learn about options for remote access to JSTOR.

Literature Cited

Chronology of Fluctuating Sea Levels since the Triassic

Bilal U. Haq; Jan Hardenbol; Peter R. Vail

Science, New Series, Vol. 235, No. 4793. (Mar. 6, 1987), pp. 1156-1167.

Stable URL:

<http://links.jstor.org/sici?sici=0036-8075%2819870306%293%3A235%3A4793%3C1156%3ACOFSL%3E2.0.CO%3B2-4>

Isotopic Paleoceanography of the Caribbean and East Pacific: Role of Panama Uplift in Late Neogene Time

Lloyd Keigwin

Science, New Series, Vol. 217, No. 4557. (Jul. 23, 1982), pp. 350-353.

Stable URL:

<http://links.jstor.org/sici?sici=0036-8075%2819820723%293%3A217%3A4557%3C350%3AIPOTCA%3E2.0.CO%3B2-O>

Divergence in Proteins, Mitochondrial DNA, and Reproductive Compatibility Across the Isthmus of Panama

Nancy Knowlton; Lee A. Weight; Luis Aníbal Solórzano; DeEtta K. Mills; Eldredge Bermingham

Science, New Series, Vol. 260, No. 5114. (Jun. 11, 1993), pp. 1629-1632.

Stable URL:

<http://links.jstor.org/sici?sici=0036-8075%2819930611%293%3A260%3A5114%3C1629%3ADIPMDA%3E2.0.CO%3B2-%23>

LINKED CITATIONS

- Page 2 of 2 -



Genetic and Morphological Evidence That the Eastern Pacific Damselfish *Abudefduf declivifrons* Is Distinct from *A. concolor* (Pomacentridae)

H. A. Lessios; G. R. Allen; G. M. Wellington; Eldredge Bermingham

Copeia, Vol. 1995, No. 2. (May 3, 1995), pp. 277-288.

Stable URL:

<http://links.jstor.org/sici?sici=0045-8511%2819950503%293%3A1995%3A2%3C277%3AGAMETT%3E2.0.CO%3B2-Z>

Enzymatic Amplification of β -Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia

Randall K. Saiki; Stephen Scharf; Fred Faloona; Kary B. Mullis; Glenn T. Horn; Henry A. Erlich; Norman Arnheim

Science, New Series, Vol. 230, No. 4732. (Dec. 20, 1985), pp. 1350-1354.

Stable URL:

<http://links.jstor.org/sici?sici=0036-8075%2819851220%293%3A230%3A4732%3C1350%3AEAIOBGS%3E2.0.CO%3B2-Z>

Nuclear and Mitochondrial DNA Comparisons Reveal Extreme Rate Variation in the Molecular Clock

Lisa Vawter; Wesley M. Brown

Science, New Series, Vol. 234, No. 4773. (Oct. 10, 1986), pp. 194-196.

Stable URL:

<http://links.jstor.org/sici?sici=0036-8075%2819861010%293%3A234%3A4773%3C194%3ANAMDCR%3E2.0.CO%3B2-X>

Genetic Divergence Among Fishes of the Eastern Pacific and the Caribbean: Support for the Molecular Clock

A. Thomas Vawter; Richard Rosenblatt; George C. Gorman

Evolution, Vol. 34, No. 4. (Jul., 1980), pp. 705-711.

Stable URL:

<http://links.jstor.org/sici?sici=0014-3820%28198007%2934%3A4%3C705%3AGDAFOT%3E2.0.CO%3B2-J>