Global phylogeography of mackerels of the genus *Scomber*

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Abstract.—Inter- and intraspecific genetic relationships among and within three species of mackerels of the genus *Scomber* were investigated by restriction site analysis of the whole mitochondrial (mt) DNA genome and direct sequence analysis of the mitochondrial cytochrome *b* gene. A total of 15 samples, averaging 19 individuals each, were collected from geographically isolated populations throughout the ranges of *S. scombrus* (two samples), *S. australasicus* (five samples), and *S. japonicus* (eight samples). Restriction site analysis with 12 restriction enzymes revealed substantial genetic variation within each species. Sample haplotype diversities ranged from 0.28 to 0.95, and nucleotide sequence diversities from 0.13% to 0.76%. Spatial partitioning of genetic variation was observed in each of the species. Eastern and western North Atlantic samples of *S. scombrus* exhibited significant heterogeneity in the distribution of mtDNA haplotypes, but no fixed restriction site differences were observed between samples. Similarly, no fixed restriction site differences occurred among samples of *S. japonicus* in the Atlantic Ocean, although there were significant differences in the distribution of haplotypes among samples. In contrast, samples of *S. japonicus* from within the Pacific Ocean were characterized by fixed restriction site differences. North and South Pacific samples of *S. australasicus* were highly divergent, and one of two divergent mtDNA matrilines was restricted to samples from the South Pacific. A 420-bp segment of the cytochrome *b* gene was sequenced for representatives of each of the major mtDNA lineages identified by restriction site analysis. *Scomber scombrus* differed from *S. australasicus* and *S. japonicus* by more than 11% net nucleotide sequence divergence, considerably greater than the 3.5% sequence divergence between *S. australasicus* and *S. japonicus*. Levels of interspecific genetic divergences based on restriction site data were similar in pattern, but were approximately 20% lower in magnitude when based on the cytochrome *b* sequences. Parsimony analysis and neighbor-joining of restriction site data, and parsimony analysis of cytochrome *b* sequences showed similar paraphyletic patterns in both *S. japonicus* and *S. australasicus*. Levels of divergence among samples of *S. japonicus* were similar to those between samples of *S. australasicus* and *S. japonicus*. Complete partitioning of halpotypes among some samples of *S. japonicus* that are morphologically distinct suggests that Atlantic and Indo-Pacific populations of *S. japonicus* may need to be recognized as separate species.

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Population structure is largely influenced by the biological characteristics of a species and the attributes of its environment that promote or impede gene flow. In marine fishes, dispersal of planktonic early life history stages and adult vagility can facilitate genetic exchange over great distances (Rosenblatt, 1963; Shulman and Bermingham, 1995). Features of the marine environment that limit gene flow are often equally large in scale, such as current systems, major changes in temperature or salinity, or the presence of large land masses (Sinclair, 1988). Accordingly, many genetic analyses of broadly distributed marine fishes have shown little divergence among conspecific samples from geographically distant locations.

Limited population structure has been demonstrated for a variety of marine fishes. Electrophoretic analyses of allozymes have revealed little population divergence among collections of milkfish (*Chanos chanos*) separated by up to 10,000 km (Winans, 1980), damselfish, *Stegastes fasciolatus*, sampled from throughout the 2500 km Hawaiian archipelago (Shaklee, 1984), in twelve species of tropical marine shore fishes sampled from both sides of the Pacific Barrier, a 5000-km expanse of deep ocean separating central and eastern Pacific shallow water habitats (Rosenblatt and Waples, 1986), or in five species of damselfishes collected from isolated Caribbean reefs (Lacson, 1992). Similarly, analyses of mitochondrial DNA have revealed little genetic differentiation among populations of five species of tropical reef fishes sampled from throughout the Caribbean (Shulman and Bermingham, 1995), or within three cosmopolitan species of tunas (Graves et al., 1984; Graves and Dizon, 1989; Scoles and Graves, 1993).

Much less information is available on the population structure of broadly distributed marine species that occur in fragmented or disjunct distributions. Recent studies of striped mullet (*Mugil cephalus*,

Crosetti et al., 1994) and bluefish (*Pomatomus saltatrix*, Goodbred and Graves, 1996) have demonstrated that significant phylogeographic population structuring can exist in discontinuously distributed marine fishes. The reduced gene flow among isolated populations of such species provide excellent opportunities for studying the effects of historical marine zoogeographical processes and observe incipient speciation.

Mackerels of the genus *Scomber*, like the striped mullet and bluefish, are ideally suited for studies of genetic population structure in cosmopolitan fishes with fragmented distributions and for comparative studies that describe genetic patterns of other more vagile scombrids (Graves et al., 1984; Graves and Dizon, 1989; Scoles and Graves, 1993). Distributional patterns of scombrids vary widely from world-wide species such as the yellowfin tuna, albacore, and skipjack tunas to supposedly wide-spread species such as the chub mackerel, *S. japonicus*, which is divided into geographically disjunct populations, to those with limited ranges such as a Spanish mackerel (*Scomberomorus munroi*) found only in the Gulf of Papua between Australia and New Guinea (Collette and Nauen, 1983). The three species of *Scomber* (*S. japonicus, S. australasicus, and S. scombrus*) occur in temperate to subtropical waters, and two (*S. japonicus* and *S. australasicus*) display antitropical distributions. Each species occurs in disjunct populations of various sizes, and their distributions overlap in several areas (Fig. 1; Collette and Nauen, 1983). The cosmopolitan chub mackerel, *S. japonicus*, occurs in coastal regions and adjacent seas of the Atlantic, Pacific, and northwest Indian oceans. The spotted chub mackerel, *S. australasicus*, is restricted to the Pacific Ocean, southeastern Indian Ocean, and the Red Sea. The Atlantic mackerel, *S. scombrus*, is restricted to the North Atlantic Ocean. The present study emphasizes *S. japonicus*, which of the three species is the most widely distributed and morphologically divergent among its isolated populations (Matsui, 1967).

Life histories and population structures

The spawning behavior and duration of the larval stage varies among the three species of *Scomber*. *Scomber scombrus* is capable of spawning serially up to 30 times in a spawning season (Watson et al., 1992) at any time of day (Walsh and Johnstone, 1992), where *S. japonicus* spawns on average only 9 times in a season, and does so only at night (Watanabe,

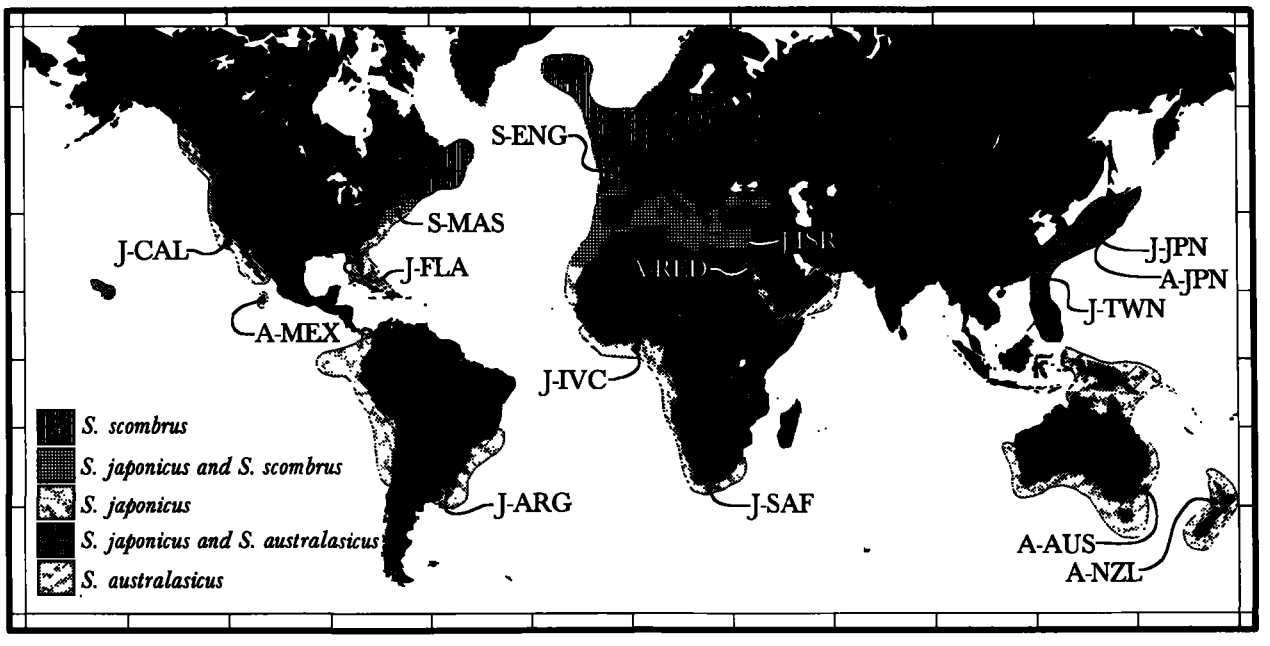

Figure 1

Sampling and distributions of Atlantic mackerel, *Scomber scombrus* (S-MAS and S-ENG), spotted chub mackerel, *S. australasicus* (A-RED, A-JPN, A-AUS, A-NZL, and A-MEX), and chub mackerel, *S. japonicus* (J-JPN, J-TWN, J-CAL, J-HAW, J-FLA, J-ARG, J-ISR, J-IVC, and J-SAF), according to Collette and Nauen (1983). *S. japonicus* distributions off Brazil and Namibia follow Perrotta and Aubone (1991) and Zenken and Lobov (1989), respectively.

1970). *Scomber japonicus* and *S. scombrus* have similar larval stage durations: eggs of *S. scombrus* and *S. japonicus* hatch in less than 6 d, and schooling behavior begins when larvae metamorphose (about 15 mm) which occurs at 24–9 d and 22–9 d, respectively (Hunter and Kimbrell, 1980; Ware and Lambert, 1985). Thus the duration of passive planktonic transport of the early life history stages is usually 29 d or less, but probably extends into the early juvenile stages as well. Little spawning or larval ecology data are available for *S. australasicus*, but it is expected that it has a similar early life history.

Exchange between regional populations of *S. scombrus* in the North Atlantic appears sufficient to maintain genetic homogeneity. Two spawning groups in the northwest Atlantic, the "northern contingent" in the southern Gulf of St. Lawerence, and the "southern contingent" between Cape Cod and Cape Hatteras, were identified by Sette (1950) by size composition and tagging data. However, these groups were not discriminated by meristic or growth character analysis (MacKay and Garside, 1969; Simard et al., 1992), or by allozyme analysis (Maguire et al., 1987). In the northeast Atlantic, two spawning groups, the "western stock" south and west of the British Isles, and the "North Sea stock," were identified by tagging (Hamre, 1980), but these groups were not distinguished by allozymic differences (Jamieson and Smith, 1987).

Matsui (1967) showed greater phenotypic variation in *S. japonicus* than in the other two species of *Scomber*, possibly because of its wider distribution. Populations of *S. japonicus* from the eastern and western Atlantic had nonoverlapping distributions of gill-raker counts, and other variable morphological characters of Atlantic populations were similar, including belly spots, strongly crenulated teeth, and large scales. In contrast, Pacific *S. japonicus* exhibited lightly crenulated teeth, no belly spots, and smaller scales (Matsui, 1967). Analysis of four polymorphic allozyme loci revealed no divergence among samples of *S. japonicus* from the southeast Atlantic off Namibia (Zenkin and Lobov, 1989), but heterogeneity in immunological reactivity suggested population structure off the northwest African coast (Weiss, 1980). A study of 14 polymorphic allozyme loci showed significant differences between samples from the north- and southeastern Pacific Ocean, which suggested reduced gene flow across the tropics in comparison with other similarly distributed pelagic fishes (Stepien and Rosenblatt, 1996). Differences in growth rates and morphological characters among samples within the southwest Atlantic led Perrotta (1993) to conclude that *S. japonicus* populations within the region had diverged to the level of subspecies.

To evaluate the genetic relationships among the three species of *Scomber*, and the discontinuous populations within them, we examined mitochondrial (mt) DNA restriction sites, and cytochrome *b* sequences. Analysis of mtDNA has proven useful in revealing phylogeographic structure in a variety of marine and freshwater fish species (Avise, 1992). Because mtDNA is clonally inherited, information regarding historical phylogenetic relationships is retained, hence analysis of mtDNA can render considerable information on historical relationships among populations and the mtDNA lineages they possess.

Materials and methods

Specimen collection

Samples of 15 to 21 individuals each of *S. scombrus*, *S. australasicus*, and *S. japonicus* were obtained from 15 locations (Table 1, Fig. 1). Specimens of *Rastrelliger kanagurta*, a species of the sister group to *Scomber* (Collette et al., 1984), were obtained from Sri Lanka. Whole fish were frozen after collection and shipped to the laboratory on dry ice.

MtDNA preparation and analysis

MtDNA-enriched genomic DNA was isolated from 3 g of lateral red muscle, or whole young-of-year fish with digestive tracts removed (A-NZL only) according to Chapman and Powers (1984) method, modified by the omission of sucrose step gradients and the use of 1.5% sodium dodecyl sulfate for mitochondrial lysis. DNAs were digested with 10 hexameric (*Apa*I, *Bgl*I, *Bsu*36I, *Dra*I, *Pvu*II, *Sca*I, *Stu*I, *Ssp*I, *Hpa*I, *Spe*I) and 2 multi-hexameric (*Ava*I, *Hae*II) restriction endonucleases following manufacturers' (Stratagene and Gibco-BRL) instructions. DNA fragments were separated by electrophoresis in 1.0 or 1.5% agarose gels, transferred to nylon membranes by Southern transfer, and hybridized to a biotin-labeled probe as described previously (Scoles and Graves, 1993). The probe was made by separately cloning four yellowfin tuna, *Thunnus albacares*, mtDNA fragments that cover the entire mitochondrial genome in the *Pst*I site of pBluescript SK- (Stratagene). MtDNA fragments were visualized by using the BluGene Non-Radioactive Nucleic Acid Detection Kit (Gibco-BRL).

A 12-letter composite mtDNA haplotype, indicating the fragment pattern for each enzyme, was developed for each individual. Letters were assigned to restriction fragment patterns as they were encountered, beginning with 'A' for the closely related

Table 1

Scomber japonicus, *S. australasicus*, and *S. scombrus*. Sample name, size, location, haplotype diversity (h), and percent nucleotide sequence diversity (π).

Sample name	Sample size (n)	Sample location	Haplotype diversity (h)	Percent nucleotide sequence diversity (π)
Scomber scombrus				
S-MAS	20	Boston, Massachusetts	0.85	0.29
S-ENG	20	Plymouth, England	0.28	0.07
Total	40		0.58	0.18
Scomber australasicus				
A-RED	15	Sinai Peninsula south, Red Sea	0.95	0.41
A-AUS	18	New South Wales, Australia	0.86	0.75
A-NZL	19	Wellington, New Zealand	0.75	0.77
A-JPN	21	Tokyo, Japan	0.81	0.30
A-MEX	20	Revillagigedo Islands, Mexico	0.59	0.13
Total	93		0.90	2.18
Scomber japonicus				
J-FLA	20	Panama City, Florida	0.93	0.40
J-ARG	18	Mar del Plata, Argentina	0.90	0.50
J-ISR	20	Mediterranean coast of Israel	0.90	0.38
J-IVC	20	Abidjan, Ivory Coast	0.91	0.39
J-SAF	20	Cape Town, South Africa	0.81	0.38
J-TWN	20	Kaohsing, Taiwan	0.86	0.29
J-JPN	20	Tokyo, Japan	0.88	0.35
J-CAL	20	San Diego, California	0.64	0.14
Total	158		0.95	2.42
Grand total	291			

S. japonicus and *S. australasicus* group, and proceeding through the alphabet, and beginning with 'Z' and proceeding in reverse alphabetical order for *S. scombrus*. For the few *S. scombrus* restriction fragment patterns that also occurred in *S. japonicus* or *S. australasicus*, the latter species' restriction morph letter designation was used.

Estimates of nucleotide sequence divergence (d) among haplotypes were determined by using the approach of Nei and Li (1979) for fragment data, and Nei and Tajima (1981) and Nei and Miller (1990) for site data, with weighting based on the proportion of fragments or sites produced by each enzyme class (Nei and Tajima, 1983). Estimates of nucleon or haplotype diversity (h) and nucleotide sequence diversity (π) were then calculated with the program DA of the statistical package REAP (McElroy et al., 1992). Corrected nucleotide sequence divergences (δ) among populations were calculated by using the protocol of Nei and Li (1979). The homogeneity of haplotype distributions among samples was evaluated by chi-square analyses using the Monte-Carlo method of Roff and Bentzen (1989) with 1000 randomizations of the data with REAP.

Restriction sites were inferred from completely additive fragment patterns, and the information for each individual was coded using a presence-or-absence matrix. Homology of restriction sites could not be assured between *S. scombrus* and the *S. australasicus-S. japonicus* group owing to the relatively large genetic divergence between the species. Restriction site data were therefore evaluated only for phylogenetic relationships within the *S. australasicus-S. japonicus* group, and an estimate of divergence between *S. scombrus* and the pooled data of *S. australasicus* and *S. japonicus* was determined by using restriction fragment data. Relationships among haplotypes were inferred by cluster analysis (unweighted pair-group method, UPGMA, Norusis, 1988). Neighbor-joining, parsimony analyses and consensus trees were generated with the PAUP* software program (test version).

DNA amplification and sequence analysis

Individuals representative of the major *Scomber* mtDNA matrilines, identified by restriction site analysis, and of *Rastrelliger kanagurta*, were selected for DNA sequence analysis. A 418-bp region of the cytochrome *b* gene was amplified from mtDNA-enriched genomic DNA isolations by the polymerase chain reaction (PCR) with primers L15079

(5'GAGGCCTCTACTATGGCTCTTACC3') and H15497 (5'GCTAGGGTATAATTGTCTGGGTCGCC3') developed by Finnerty and Block (1992) for blue marlin (*Makaira nigricans*). Double-stranded DNA amplifications were accomplished in 100 µl of 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 0.2 mM of each dNTP, 50 pmol of each primer, 50 ng genomic DNA, and 2.5 units *Taq* polymerase (Perkin-Elmer/Cetus). Cycling parameters were preceded by a 4-min initial denaturation at 94°C and included 38 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min, followed by a 5-min final extension at 72°C. Primers L15079 and H15497 would not amplify some haplotypes of *S. australasicus*. Specific primers with conserved 3' ends were designed from other sequences: CYT2A (5'TACCTTTTCATGGAAACATG3') and CYT2B (5'AAGAGGTTGGGAGAGAAGA3'). Both strands were fully sequenced by using the Sequenase kit (United States Biochemical) or the CircumVent Cycle Sequencing Kit (New England BioLabs) following the vendors' protocols.

Sequences were aligned by eye to the human mitochondrial cytochrome *b* sequence[1] (Anderson et al., 1981). Nucleotide sequence divergences (d) among cytochrome *b* sequences were generated with the program K of REAP. Cytochrome *b* sequences were analyzed by parsimony analysis with rooting to *Rastrelliger kanagurta* by using ALLTREES in PAUP.

Results

MtDNA restriction site analysis of 40 *Scomber scombrus* from two locations revealed a total of 56 restriction sites, of which 16 were polymorphic, defining 13 haplotypes. Nucleotide sequence divergences among the haplotypes of *S. scombrus* ranged from d=0.17% to 0.86%. An average of 294 bp was surveyed, representing 1.75% of the mitochondrial genome. The average size of the *S. scombrus* mtDNA genome, determined from several restriction fragment profiles from each of the 12 restriction enzymes, was 16,784 ±213 bp (SD).

Restriction site analysis of 246 *S. japonicus* and *S. australasicus* revealed a total of 93 unique haplotypes, including information from 86 restriction sites, of which 58 were polymorphic. Among haplotypes of *S. japonicus* and *S. australasicus*, nucleotide sequence divergences ranged from d=0.15% to 2.9%. On average, 310 bp were surveyed per individual, representing 1.85% of the mtDNA genome. The average size of the mtDNA genome of *S. japonicus* or *S. australasicus* was estimated to be 16,781 ±195 bp (SD), not statistically different than that of *S. scombrus*.

Genetic diversities

MtDNA restriction site analysis revealed that the genetic variability was lower in *S. scombrus* than the other two species (Table 1). S-ENG was the least variable sample in the study (h=0.28), consisting of four haplotypes of which three occurred only once (Table 2). Ten haplotypes were revealed in S-MAS, with three at elevated frequencies, resulting in a haplotype diversity of h=0.85. The overall haplotype diversity for *S. scombrus* was h=0.58, and nucleotide sequence diversity was π=0.18%. Three restriction enzymes, *Pvu*II, *Bgl*I, and *Bsu*36I, were invariant in *S. scombrus*, whereas the remaining nine restriction enzymes each revealed from two to five fragment patterns.

A wide range of diversities was observed among samples of *S. australasicus* (h=0.59–0.95) and *S. japonicus* (h=0.64–0.93) based on the restriction site data. Eastern Pacific samples of *S. australasicus* and *S. japonicus* from Mexico (A-MEX, h=0.59, π=0.13%) and California (J-CAL, h=0.64, π=0.14%) had lower diversities than other samples, possessing only four and six haplotypes, respectively (Tables 1 and 2). The highest haplotype diversity occurred in A-RED (h=0.95), with five of 10 haplotypes represented twice; the highest nucleotide sequence diversities occurred in A-NZL (π=0.77%) and A-AUS (π=0.75%) owing to the presence of two divergent mtDNA matrilines within each sample (Tables 1 and 2). With the exception of *Bgl*I in *S. australasicus*, all restriction enzymes were variable in both species, revealing two to seven fragment patterns in *S. australasicus*, and two to 15 in *S. japonicus*. The enzyme *Apa*I revealed the greatest number of variants in both species.

Genetic divergences

Restriction fragment analysis showed *S. scombrus* to be highly divergent from *S. australasicus* and *S. japonicus*, with a net nucleotide sequence divergence of δ=11.9% between *S. scombrus* and the pooled data of *S. japonicus* and *S. australasicus*. In comparison, the mean net nucleotide sequence divergence between *S. japonicus* and *S. australasicus* based on the restriction site data was only δ=1.17%. Probabilities of Roff and Bentzen chi-square tests among samples that shared haplotypes are given in Table 3.

Scomber scombrus Considerable intraspecific divergence was evident in *S. scombrus* from the mtDNA

[1] GenBank accession no. V00662.

Table 2

Distributions of mtDNA haplotypes among samples of species of *Scomber*, grouped by their occurrence in phenetic cluster analysis. Letters represent fragment patterns produced by the following enzymes (left to right): *Sca*I, *Dra*I, *Stu*I, *Pvu*II, *Hae*II, *Apa*I, *Ava*I, *Ssp*I, *Bgl*I, *Hpa*I, *Spe*I, *Bsu*36I.

Western Pacific *Scomber japonicus*

ID	Haplotype	J-TWN	J-JPN	Total
1	BAIABIAGEBAA	4	5	9
2	BAIABLADEBAA	5	4	9
3	BAIABMAGEBAA	5	4	9
4	BAIABJADEBAA	2	1	3
5	BAIABMHGEBAA		2	2
6	BAIABJAGEBAA		1	1
7	BAIABLAGEBAA	1		1
8	BAIABMADEBAA	1		1
9	BAIABMAGEBCA		1	1
10	BAIABNAGEBAA		1	1
11	BAIABOAEEBAA		1	1
12	BAIABOAGEBAA	1		1
13	BAIAGIAGEBAA	1		1

Eastern Pacific *Scomber japonicus*

ID	Haplotype	J-CAL	Total
14	BAIABIADEAAA	12	12
15	BAIABJADEAAA	5	5
16	BAIABIADEAAB	1	1
17	BAIABIDDEAAA	1	1
18	BAIADIADEAAA	1	1
19	BAJABIADCAAA	1	1

Atlantic *Scomber japonicus*.

ID	Haplotype	J-FLA	J-ARG	J-SAF	J-IVC	J-ISR	Total
20	ABAAABAAAAAA	1		1	3	1	6
21	AAAAABADAAAA	1			1		2
22	ABAAABAHAAAA		3				3
23	ABAAABADAAAA					2	2
24	ABAAABAIAAAA		1				1
25	ABAAADAAAAAA			1			1
26	ABAAAKADAAAA					1	1
27	ABAAEBAAAAAA				1		1
28	ABAAEBADAAAA					1	1
29	ABABABAHAAAA		1				1
30	AAAAABAAAAAA	1					1
31	AAAAAAAAAAAA	6	4		3	2	15
32	AABAAAAAAAAA	4					4
33	AAAAAJAAAAAA		2				2
34	AAAAAAABAAAA				1		1
35	AAAAAPAAAAAA		1				1
36	AAAAAABAABAA			1			1
37	AABAAAAABAAA	1					1
38	AABAAAACAAAA	1					1
39	ABAAAAAAAAAA	1					1
40	BAAAAJAAAAAA		1				1
41	AAGAAAADAAAA		4	9	5	6	24
42	ABGAAAADAAAA			1	2	3	6
43	AAAAAAADAAAA	2		1	1		4

continued on next page

Table 2 (continued)

Atlantic *Scomber japonicus*. (continued)

ID	Haplotype	J-FLA	J-ARG	J-SAF	J-IVC	J-ISR	Total
44	AAGAAAADADAA				1	1	2
45	AAAAAAADAEAA		1				1
46	AAAAAEADAAAA			1			1
47	AAAAAGGDAAAA					1	1
48	AABAACADAAAA	1					1
49	AACBABABAAAA	1					1
50	AAGAAAADBAAA				1		1
51	AAGAAAAEAAAA			1			1
52	AAGAAAAEABAA			1			1
53	AAGAABADAAAA			1			1
54	AAHAAAADAAAA			1			1
55	ABAAAAADAAAA					1	1
56	ABAAAAADAAAB			1			1
57	ABAAAAADBAAA				1		1
58	BAAAAAADAAAA					1	1

Red Sea *Scomber australasicus*

ID	Haplotype	A-RED	Total
59	BADAAAADDEAA	2	2
60	BADAAAADDFAA	2	2
61	BADAFAADAFAA	2	2
62	BADBAAAAAFAA	2	2
63	BADBAAADAFAA	2	2
64	AADBAAADAFAA	1	1
65	BADAAAADAEAA	1	1
66	BADBABADAFAA	1	1
67	BALBAAADAFAA	1	1
68	BALBAJADAFAA	1	1

Unique lineage *Scomber australasicus*

ID	Haplotype	A-NZL	A-AUS	Total
69	BCDBBAADABAA	8	6	14
70	BCDBBAADAAAA		1	1
71	BCDBBAEDABAA		1	1
72	BCDBBAFDABAA		1	1
73	BCDBBBADABAA	1		1
74	BCDBBGADABAA	1		1
75	BCDBBHADABAA		1	1
76	BCDBCAADABAA	1		1
77	BDDBBAADABAA		1	1
78	BDDBBAADABBA		1	1

Ubiquitous lineage *Scomber australasicus*

ID	Haplotype	A-NZL	A-AUS	A-MEX	A-JPN	Total
79	BAEABACFABAA	1	1	12	9	23
80	BAEABACEACAA	6	4			10
81	BBEABACFABAA			2	1	3
82	BAEABACFABAA			5		5
83	BAKABACFABAA				3	3
84	BAEABJCFABAA				2	2

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Table 2 (continued)

Ubiquitous lineage *Scomber australasicus* (continued)

ID	Haplotype	A-NZL	A-AUS	A-MEX	A-JPN	Total
85	BAEABABFABAA			1		1
86	BAEABACFAFAA				1	1
87	BAEABSCFABAA				1	1
88	BAEBBACFABAA				1	1
89	BAFABACEACAA	1				1
90	BBDABACFABAA		1			1
91	BBEBBRCFABAA				1	1
92	BEKBBACFABAA				1	1
93	CAEABACFABAA				1	1

Scomber scombrus

ID	Haplotype	S-ENG	S-MAS	Total
94	ZZZZZZZZZFZZ	17	7	24
95	ZZZZYZZZZFZZ		4	4
96	ZZZZYZZZZFZZ		2	2
97	CZYZZXZZZFZZ		1	1
98	XZZZZZZZZFYZ		1	1
99	ZXZZZZZZZFZZ	1		1
100	ZYZZYZZZZFZZ		1	1
101	ZZZZXZZZZFZZ		1	1
102	ZZZZZVYZZFZZ	1		1
103	ZZZZZWZZZFZZ		1	1
104	ZZZZZZZXZFZZ	1		1
105	ZZZZZZZYZFZZ		1	1
106	ZZZZZZZZZYZZ		1	1

restriction site data. Only one *S. scombrus* haplotype (no. 94) was shared between the eastern and western North Atlantic samples, and it occurred at significantly different frequencies in each (0.35 in S-ENG, and 0.85 in S-MAS, Table 2). The distribution of haplotypes between the two samples was highly heterogeneous ($P<0.001$), although the estimate of net nucleotide sequence divergence between S-ENG and S-MAS was low ($\delta=0.011\%$), reflecting the close relationship among haplotypes (Fig. 2).

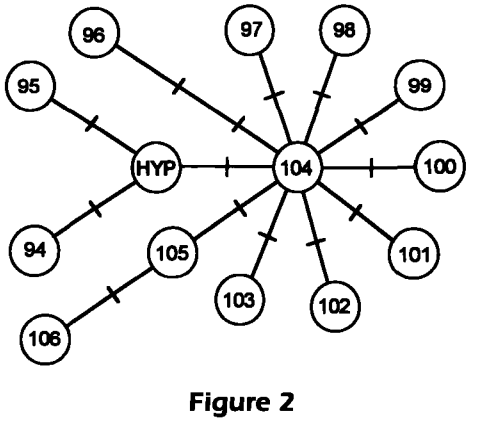

Figure 2

Parsimony network illustrating the relationship among mtDNA haplotypes of *Scomber scombrus*. One hypothetical haplotype (HYP) is indicated. Each dash corresponds to a single restriction-site gain or loss.

Scomber australasicus Restriction site analysis of *S. australasicus* mtDNA exhibited a range of intraspecific divergences. Samples collected from Australia and New Zealand were very similar. Three haplotypes (nos. 69, 79, and 80), representing two genetically divergent matrilines, occurred at similar frequencies in each sample (Table 2), and no heterogeneity was revealed ($P=0.718$, $\delta=-0.019\%$). The two samples of *S. australasicus* from the North Pacific (A-JPN and A-MEX) revealed greater divergence, comprising twelve haplotypes, of which two were shared, and one (no. 79) occurred in a large number of individuals in both samples ($n=9$ and $n=12$, respectively). Haplotype 82 occurred in five individuals from A-MEX but was not present in the A-JPN

Table 3

Probabilities of significance from Roff and Bentzen's (1989) chi-square analysis with 1000 randomizations of the data of samples of Atlantic *Scomber japonicus*, Pacific *S. japonicus*, *S. australasicus*, and *S. scombrus* that shared haplotypes.

Comparison	χ^2	Number of simulations exceeding χ^2	Probability
Atlantic *Scomber japonicus*			
J-ISR J-IVC J-SAF J-FLA J-ARG	191.36	0	<0.001**
J-ISR J-IVC J-SAF	48.70	451	0.451^{NS}
J-ARG J-IVC J-SAF	60.84	28	0.028*
J-ARG J-IVC	22.21	68	0.070^{NS}
J-ARG J-SAF	26.89	0	0.001**
J-FLA J-ARG	28.37	1	0.001**
J-FLA J-ISR J-IVC	58.40	16	0.016*
J-FLA J-ISR	32.00	1	0.001**
J-FLA J-IVC	24.33	14	0.014*
Pacific *Scomber japonicus*			
J-JPN J-TWN	10.67	658	0.658^{NS}
Scomber australasicus			
A-JPN A-MEX A-AUS A-NZL	128.94	0	<0.001**
A-JPN A-MEX	17.75	13	0.013*
A-AUS A-NZL	11.67	718	0.718^{NS}
Scomber scombrus			
S-ENG S-MAS	20.17	0	<0.001**

*Significantly different at α=0.05.
**Significantly heterogeneous at α=0.01.
^{NS}Not significant.

Scomber japonicus Of the three species, samples of *S. japonicus* spanned the greatest geographical area, and mtDNA restriction site analysis of this species revealed the largest range of divergences. In the North Pacific, samples from Japan (J-JPN) and Taiwan (J-TWN) shared four haplotypes, three of which occurred in more than one individual in each sample (nos. 1–3, Table 2), and no significant heterogeneity was observed between the samples (P=0.658, α=0.05, δ=–0.010%). In contrast, a comparison of the pooled data of J-JPN and J-TWN with *S. japonicus* from the eastern North Pacific (J-CAL) revealed one fixed restriction site difference, a net nucleotide sequence divergence of δ=0.30%, and genotype distributions were significantly different (P<0.001, α=0.05).

Many closely related haplotypes were observed in samples of *S. japonicus* from the Atlantic and Mediterranean Sea, and several were shared among two or more sample locations (Table 2). No significant differences were observed in the distribution of haplotypes among J-ISR, J-IVC, and J-SAF, from the eastern Atlantic (P=0.451, α=0.05, δ=–0.003%–0.015%, Table 4), or between J-IVC and J-ARG across the Atlantic (P=0.07, α=0.05, δ=0.025%). All other tests of haplotype frequencies among Atlantic samples were significant (P<0.05).

sample. Although the net nucleotide sequence divergence between A-JPN and A-MEX was small (δ=0.021%), the distribution of haplotypes between the samples was significantly heterogeneous (P=0.013, α=0.05). Together, the Australian and New Zealand samples of *S. australasicus* from the South Pacific were genetically distinct from the combined North Pacific samples (A-JPN and A-MEX). The mean corrected nucleotide sequence divergence between the pooled groups was high (δ=0.54%), and only one haplotype was shared between the pooled South and North Pacific samples (no. 79), occurring at very different frequencies (0.05 and 0.51, respectively).

The Red Sea sample of *S. australasicus* was distinct from all other collections of this species. The mean net nucleotide sequence divergence between A-RED and the pooled data of all other *S. australasicus* was δ=0.86%. The Red Sea sample was less divergent from the *S. australasicus* samples from Australia and New Zealand (δ=0.51%) than were the *S. australasicus* samples from Japan and Mexico (δ=1.2%)

Phylogeographic patterns

MtDNA restriction site analysis and neighbor-joining of *S. japonicus* and *S. australasicus* mtDNA haplotypes revealed five major clusters: *S. japonicus* from the Pacific Ocean, *S. japonicus* from the Atlantic Ocean, *S. australasicus* from the Red Sea, a "ubiquitous" cluster of haplotypes of *S. australasicus* from all other samples of this species, and a "unique" cluster of only New Zealand and Australia *S. australasicus* haplotypes (Fig. 3).

Parsimony analyses were used to explore cladistic relationships among the haplotypes of *S. japonicus* and *S. australasicus* identified by mtDNA restriction site analysis. Parsimony analysis among all *Scomber* haplotypes could not be conducted because *S. scombrus* mtDNA sequences were so divergent that it was not possible to determine homologous restriction site characters among the three species. Mainly interested in patterns occurring in *S. japonicus*, we chose a root in *S. australasicus* (haplotype no. 92) on the basis of results of parsimony analyses of cytochrome *b* sequences that included *S. scombrus* and a

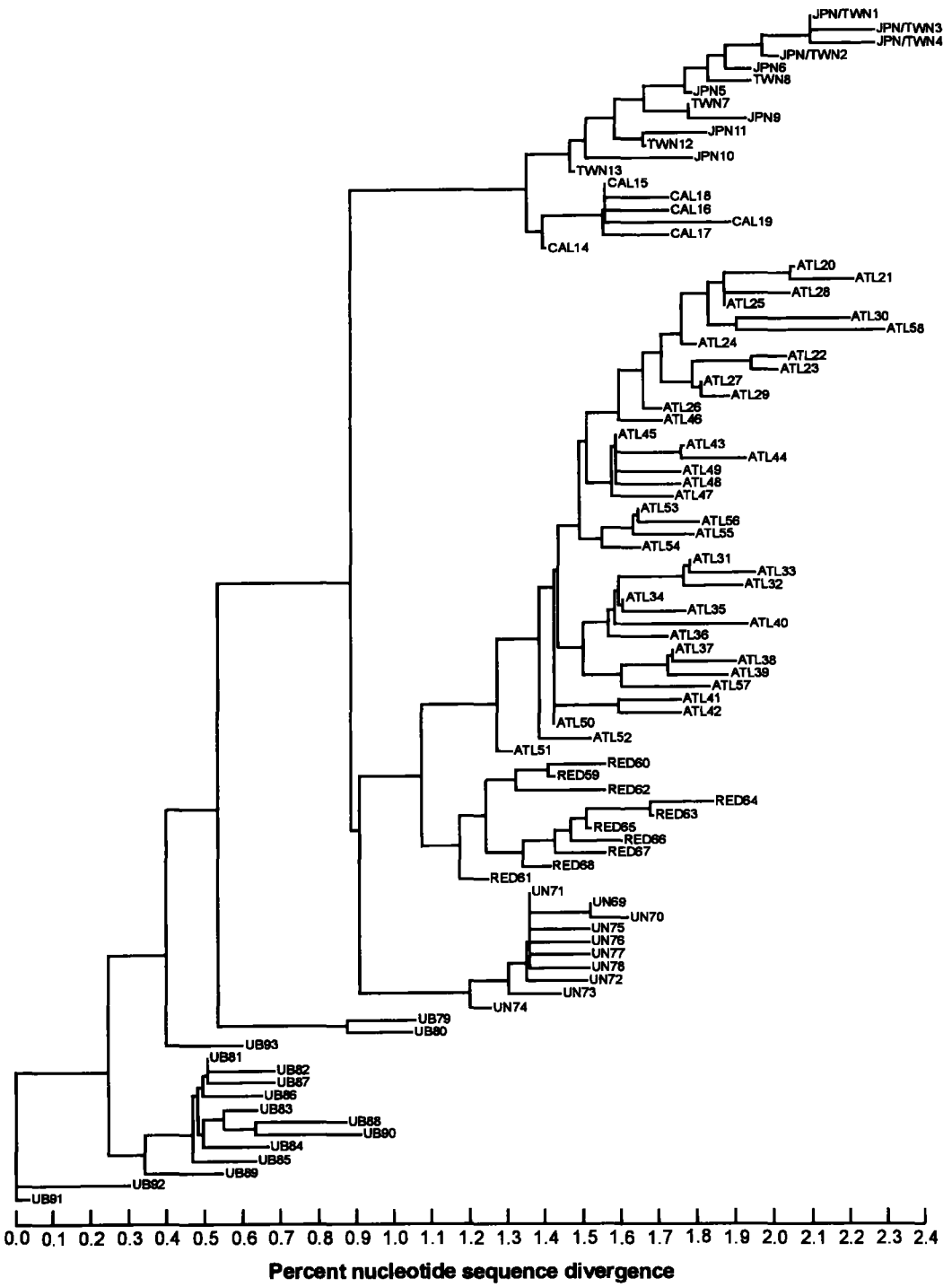

Figure 3

Neighbor-joining analysis of mtDNA haplotypes of *Scomber japonicus* and *S. australasicus* identified by restriction site analysis. Branch lengths are indicated. The terminal nodes labeled ATL are Atlantic haplotypes of *S. japonicus* 20–58 from J-FLA, J-ARG, J-SAF, J-IVC, and J-ISR. Haplotypes 1–13 labeled JPN or TWN are *S. japonicus* from J-JPN and J-TWN. Haplotypes 14–9 labeled CAL are *S. japonicus* from J-CAL. Haplotypes 61–68 labeled RED are *S. australasicus* haplotypes from A-RED. Those labeled UN are "unique" haplotypes 69–78 of *S. australasicus* from A-NZL and A-AUS. Those labeled UB are "ubiquitous" haplotypes 79–93 of *S. australasicus* from A-NZL, A-AUS A-MEX, and A-JPN.

more distant outgroup, *Rastrelliger kanagurta*, and a preliminary parsimony tree drawing. Analysis of 58 informative characters yielded 1031 equally parsimonious trees. Each of these 1031 trees shared the same topology among the five major matrilines and differed only within groups, with most rearrangements occurring among Atlantic *S. japonicus* haplotypes. Branches defining the five matrilines were supported at 100% in a majority-rule consensus that illustrated the relationship of all haplotypes of *S. japonicus* and *S. australasicus* and revealed a topology that was not different from that obtained by neighbor-joining (Fig. 4).

The Red Sea sample had haplotypes intermediate to *S. japonicus* and *S. australasicus*, highlighted by character state reversals. Two reversals occurred among the Red Sea *S. australasicus* and the Japan-Taiwan *S. japonicus* clades, and the unique clade of *S. australasicus*. An *Hpa*I site that is absent in eight of the 10 Red Sea haplotypes (nos. 60, 62–68), nine of the 10 unique *S. australasicus* haplotypes (nos. 69–73, 75–78), and all of the *S. japonicus* haplotypes from Japan and Taiwan, is present in all other haplotypes. A *Pvu*II site that is absent in six of the 10 Red Sea haplotypes (nos. 63–68) and all haplotypes of the unique *S. australasicus* clade is present in all other haplotypes. Consequently, corrected nucleotide sequence divergences among the Red Sea and unique *S. australasicus* haplotypes are lower than those among Red Sea and Atlantic *S. japonicus* haplotypes (Table 4). Although UPGMA cluster analysis grouped the Red Sea haplotypes with the unique *S. australasicus* haplotypes (data not shown), neighbor-joining resulted in the placement of the Red Sea sample with Atlantic *S. japonicus*, concordant with the majority-rule consensus. Neighbor-joining is favored over UPGMA because it removes the assumption that the data are ultrametric (the condition that all the taxa are equidistant from the root) (Swofford and Olsen, 1990).

Parsimony analysis of the cytochrome *b* sequences (Table 5) yielded a tree topology that was similar to the patterns observed by parsimony and neighbor-joining of mtDNA restriction site data of *S. japonicus* and *S. australasicus*. However, the analysis showed greater similarity between the unique and ubiquitous *S. australasicus* clades that were not separated by Pacific *S. japonicus*. A majority-rule consensus of two equally parsimonious trees of 86 informative characters (of 93 variable positions) of cytochrome *b* sequences showed that *S. scombrus* is the sister group to *S. japonicus* and *S. australasicus* (Fig. 5). Sequence divergences among the cytochrome *b* sequences were 1.7–3.1% in *S. australasicus*, 0.8–4.3% in *S. japonicus*, and 2.8% in *S. scombrus*. The cytochrome *b* divergences were 2.8–4.0% between haplotypes of *S. japonicus* and *S. australasicus*, and ranged from 14.6 to 17.6% between the *S. scombrus* and the *S. japonicus-S. australasicus* group. The divergence of cytochrome *b* haplotypes between *Rastrelliger kanagurta* and *S. scombrus* was 21.5% and between *Rastrelliger kanagurta* and the *S. japonicus-S. australasicus* group was 17.1–18.8%. Neighbor-joining analysis of divergences among the cytochrome *b* sequences revealed a tree that had equal topology to that in Fig. 5 (data not shown). Parsimony and neighbor-joining of mtDNA restriction site data showed a paraphyletic pattern in both *S. japonicus* and *S.*

Table 4

Percent net nucleotide sequence divergences (δ) among samples of *Scomber australasicus* and *Scomber japonicus* derived from mtDNA restriction site data.

	J-FLA	J-ARG	J-ISR	J-IVC	J-SAF	J-TWN	J-JPN	J-CAL	A-RED	A-AUS	A-NZL	A-JPN	A-MEX
J-FLA	—												
J-ARG	0.042	—											
J-ISR	0.137	0.071	—										
J-IVC	0.078	0.025	–0.003	—									
J-SAF	0.165	0.099	0.007	0.015	—								
J-TWN	1.554	1.472	1.493	1.522	1.483	—							
J-JPN	1.576	1.497	1.515	1.544	1.503	–0.010	—						
J-CAL	1.209	1.128	1.151	1.179	1.176	0.284	0.319	—					
A-RED	0.752	0.706	0.724	0.728	0.723	1.136	1.159	1.077	—				
A-AUS	0.934	0.907	0.874	0.892	0.861	0.974	0.990	0.886	0.489	—			
A-NZL	0.926	0.909	0.878	0.894	0.860	0.991	1.004	0.872	0.528	–0.019	—		
A-JPN	1.640	1.599	1.560	1.579	1.505	1.523	1.529	1.533	1.176	0.554	0.477	—	
A-MEX	1.688	1.654	1.604	1.622	1.543	1.602	1.606	1.607	1.267	0.619	0.533	0.021	—

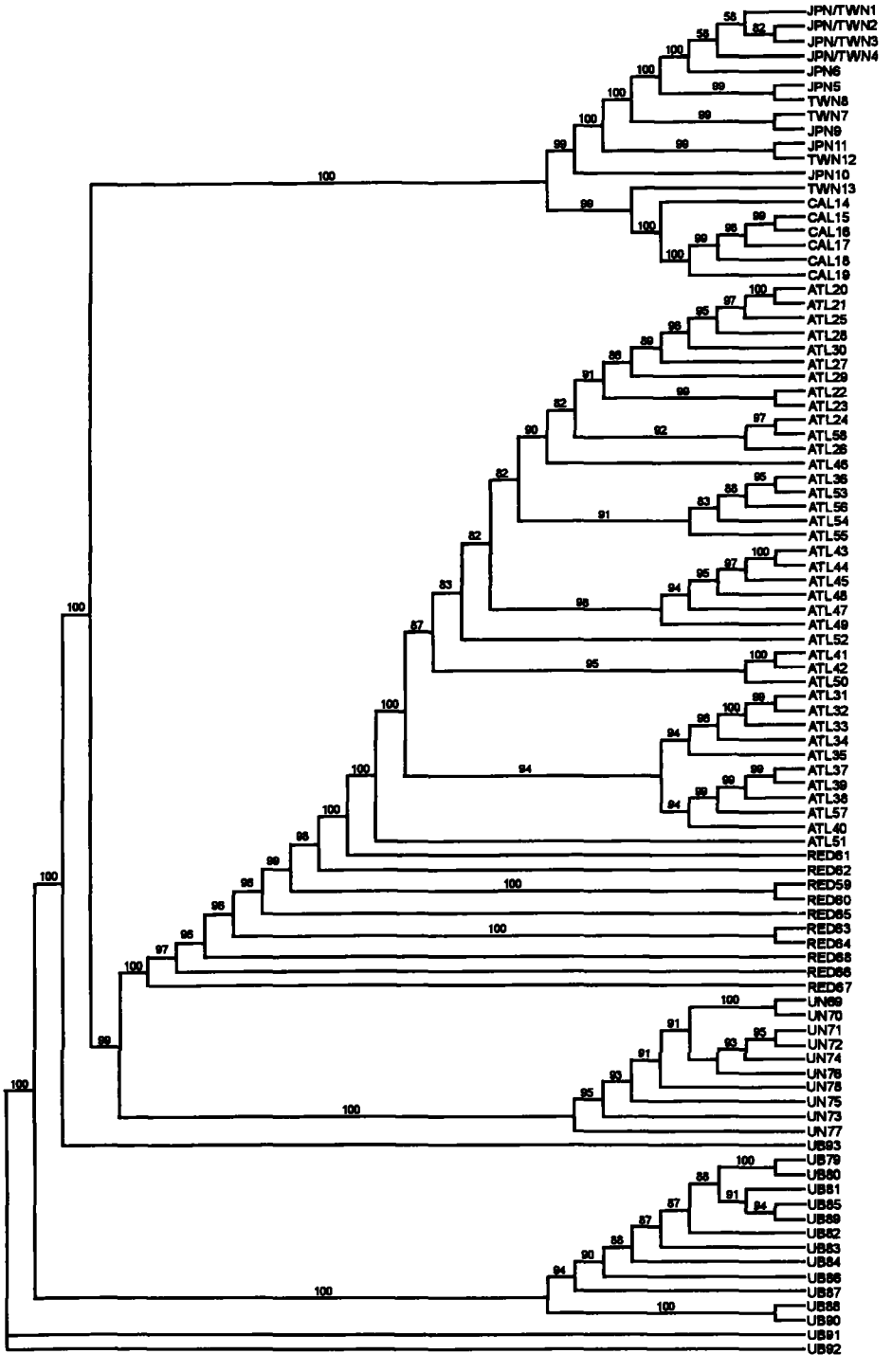

Figure 4

Majority rule consensus of 1031 equally parsimonious trees of *Scomber japonicus* and *S. australasicus* mtDNA haplotypes identified by restriction site analysis. Each of the 1031 equally parsimonious trees has the same topology among the major clades, with rearrangements only within groups. Numbers at nodes indicate the percentage of times the node was supported in 1000 bootstrap replications with a heuristic search. There were 58 informative characters used to draw the tree which is 118 steps in length. The consistency index is 0.86. Terminal nodes are labeled as in Figure 3.

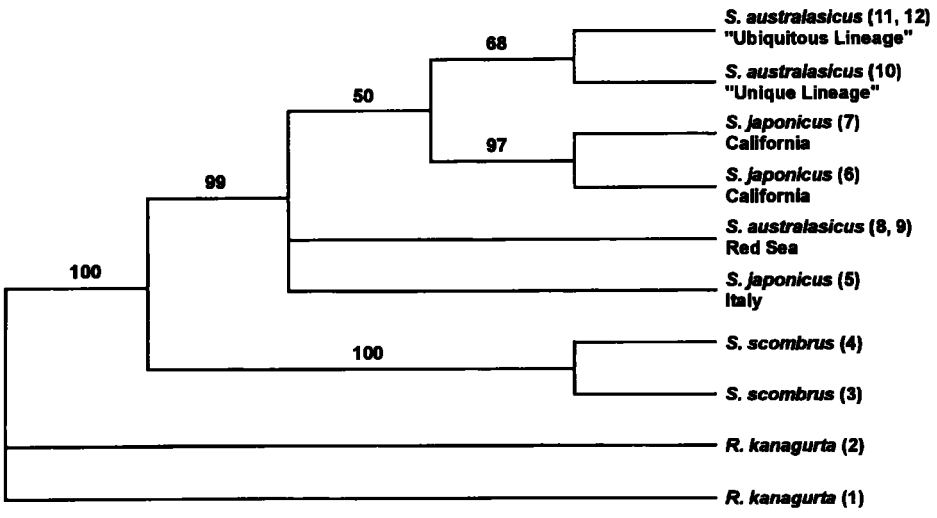

Figure 5

Majority rule consensus of two equally parsimonious trees drawn by using cytochrome *b* sequences of selected individuals of the major lineages chosen on the basis of results of parsimony and cluster analyses of restriction site data. The sequence numbers in parentheses correspond to the numbered sequences in Table 5. The tree is rooted to *Rastrelliger kanagurta* (sequence no. 2). Numbers at nodes indicate the percentage of times the node was supported in 1000 bootstrap replications with a heuristic search. There were 86 informative characters used to draw the tree which is 113 steps in length. The consistency index is 0.89.

australasicus. Parsimony analysis of cytochrome *b* sequences also revealed paraphyly in both *S. japonicus* and *S. australasicus*.

Discussion

Relationships at the generic level

Early studies of *Scomber* demonstrated considerable morphological divergence between species. Characterized by the presence of a swimbladder, *S. japonicus* and *S. australasicus* were once placed in the genus *Pneumatophorus*, leaving only *S. scombrus* in the genus *Scomber* (Starks, 1921). The two genera were subsequently united because many other morphological characters were shared among the three species (Fraser-Brunner, 1950; Matsui, 1967) and because the presence of a swimbladder can vary intraspecifically in the Scombridae (Collette and Gibbs, 1963).

Concordant with morphological dissimilarities between these two groups, the level of genetic divergence between *S. scombrus* and the *S. australasicus-S. japonicus* group estimated from mtDNA restriction fragment data was high (δ=11.2%). The divergence of these groups estimated from direct sequence analysis of the cytochrome *b* gene was also high (δ=16.7%). For comparison, we calculated divergences among 29 scombroid species and drew a phenetic tree (UPGMA), using the 600-bp cytochrome *b* sequences reported by Block et al. (1993).[2] The lowest intergeneric divergence in the tree was δ=3.0% between *Makaira* and *Istiophorus*, whereas the greatest was δ=31% between *Trichiurus* and *Gempylus*. The greatest divergence between species within a single genus was δ=14.6% between *Scomber japonicus* and *S. scombrus*, slightly lower than our estimate. Other interspecific divergences were δ=13.5% between *Scomberomorus cavalla* and *Scomberomorus maculatus*, 3.9% between *Sarda chiliensis* and *Sarda sarda*, 1.2–5% among five species of *Thunnus*, and 0.3–5.2% among five species of *Tetrapturus*. Although the clonal inheritance of mtDNA can result in high levels of divergence compared with estimates determined from nuclear DNA markers, the high values seen in *Scomber* suggest that a re-evaluation of generic taxonomy in *Scomber* may be in order.

Relationships at the species level

The current taxonomy of *Scomber* is based on multiple morphological characters, including the number of interneural bones under the first dorsal fin, length of the space between the dorsal fins in rela-

[2] GenBank accession nos. L11532-L11562.

Table 5

DNA sequences of cytochrome *b* of 12 individuals of *Scomber*, and *Rastrelliger kanagurta*, with reading frame indicated. Reported sequences of *S. japonicus* from Italy[1] and *S. scombrus*, sequences nos. 3 and 5, are from Finnerty and Block (1992). Indicated positions are relative to the full-length human mitochondrial DNA sequence[2] (Anderson et al., 1981). A '.' indicates identity to sequence number 1, and a '-' represents missing data.

	Species	GenBank	Haplotype												
								15085							15100
1	*R. kanagurta*	AF032704	Unknown		ta	gaa	aca	tga	aac	atc	gga	gtt	g--		
2	*R. kanagurta*	AF032705	Unknown												
3	*S. scombrus*	L11544	Unknown	t	g..	..g	...	...	...	g..	..t	..a	.tc		
4	*S. scombrus*	AF032706	S-MAS94		..	..g	...	...	...	g..	..t	..a	.tc		
5	*S. japonicus* (Italy)	L11546	Unknown	t	a.g	..g	...	...	...	..t	..t	..g	.tt		
6	*S. japonicus*	AF032707	J-CAL15	c	a.g	...	...	...	...	..t	..t	..g	.tt		
7	*S. japonicus*	AF032708	J-CAL14		a.g	...	...	...	...	..t	..t	..g	.tt		
8	*S. australasicus*	AF032709	A-RED62		.g	..-	...	...	...	..t	---	---	---		
9	*S. australasicus*	AF032710	A-RED60								gt	..a	.tt		
10	*S. australasicus*	AF032711	A-AUS71	c	a.g	...	...	...	...	..t	..t	..a	.tt		
11	*S. australasicus*	AF032712	A-MEX79								..t	..a	.tt		
12	*S. australasicus*	AF032712	A-MEX81								..t	..a	.tt		

	15115							15130					15145						15160
1	ctt	ctc	ctc	tta	gta	atg	ata	acc	gct	ttc	gtt	ggc	tac	gtc	ctt	ccc	tga	gga	caa	atg
2	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	..c	...	...	c.c	...	...	..g	..t	..c	...	...	...	...	...	...	...	...	...	...	...
4	..c	...	...	c.c	...	...	..g	..t	..c	...	...	...	...	...	...	...	...	...	...	...
5	...	...	...	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..t	...	...
6	...	...	...	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..c	...	...
7	...	...	...	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..c	...	...
8	---	---	---	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..t	...	...
9	...	...	...	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..t	...	...
10	...	...	...	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..c	...	...
11	...	...	...	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..c	...	..a
12	...	...	...	c.c	...	..a	...	...	..a	...	..c	...	...	...	...	...	...	..c	...	..a

	15175							15190					15205						15220
1	tcc	ttc	tga	ggt	gca	act	gtc	att	act	aac	ctc	ctt	tcc	gca	gtc	cct	tat	gta	ggc	act
2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	...	...	...	..a	..c	...	...	..c	...	...	..a	..c	..a	...	..t	..a	..c	...	...	..a
4	...	...	...	..g	..c	...	...	..c	...	...	..a	..c	..a	...	..t	..a	..c	...	...	..a
5	...	...	...	..g	..c	...	...	...	..g	...	..a	..c	..g	...	...	..a	..c	..t	..t	...
6	...	...	...	..g	..c	..c	...	...	..a	..t	..a	...	..a	...	...	..a	..c	..c	..t	...
7	...	...	...	..g	..c	..c	...	...	..a	..t	..a	...	..g	...	...	..a	..c	..c	..t	...
8	...	...	...	..a	..c	...	...	...	..a	...	..a	...	..g	...	...	..a	..c	..c	..t	...
9	...	...	...	..a	..c	...	...	...	..a	...	..a	...	..g	...	...	..a	..c	..c	..t	...
10	...	...	...	..g	..c	..c	...	...	..a	...	..a	...	..g	...	...	..a	..c	..c	..t	...
11	...	...	...	..g	..c	..c	...	...	..a	...	..a	...	..g	...	...	..a	..c	...	..t	...
12	...	...	...	..g	..c	..c	...	...	..a	...	..a	...	..g	...	...	..a	..c	...	..t	...

	15235							15250					15265						15280
1	acc	cta	gta	gaa	tga	atc	tga	ggt	ggc	ttc	tcc	gtc	gac	aat	gca	acc	ctc	act	cga	ttc
2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	..a	..c	..t	...	...	..t	...	..g	...	...	..a	..a	...	...	..t	..g	...	...	..g	...
4	..a	..c	..t	...	...	..t	...	..g	...	...	..a	..a	...	...	..t	..g	...	...	..g	...
5	..a	..c	..t	..g	...	...	...	..c	...	...	..a	..a	...	...	..c	...	...	..c	...	...
6	...	..c	..t	..g	...	...	...	..c	...	...	..a	..a	...	...	t.c	...	...	..c	...	...
7	...	..c	..t	..g	...	...	...	..c	...	...	..a	..a	...	...	..c	...	...	..c	...	...
8	..a	..c	..t	..g	...	...	...	..c	...	...	..a	..a	...	...	..c	...	...	..c	...	...
9	..a	..c	..t	..g	...	...	...	..c	...	...	..a	..a	...	...	..c	...	...	..c	...	...
10	..a	..c	..t	..g	...	...	..g	..c	...	...	..a	..a	...	...	..c	...	...	..c	...	...
11	..a	..c	..t	..g	...	...	...	..c	...	...	..a	..a	...	...	..c	...	...	..c	...	...
12	..a	..c	..t	..g	...	...	...	..c	...	...	..a	..a	...	...	..c	...	...	..c	...	...

continued on next page

tion to the dorsal groove length, and relative positions of the anal and dorsal fin origins (Matsui, 1967). Previous genetic data supported the specific status of *S. australasicus* and *S. japonicus*: allozyme electrophoresis revealed fixed allelic differences between *S. australasicus* and Pacific *S. japonicus* and pro-

Table 5 (continued)

	15295							15310					15325						15340
1	ttc	gca	ttc	cat	ttc	ctt	tac	ccg	ttc	gtc	atc	gca	gca	ata	aca	atc	ctg	cac	ctt	ctc
2	...	...	...	...	...	...	.t.	...	...	...	...	...	...	...	...	...	...	...	...	...
3	...	..c	..t	..c	...	..a	.t.	..c	..t	..t	...	tt.	..g	gc.	g.g	g.t	..c	...	..a	..a
4	...	..c	..t	..c	...	..a	.t.	..c	..t	..t	...	tt.	..g	gc.	g.g	g.t	..c	...	..a	..a
5	..t	..c	...	..c	...	..a	.t.	..a	...	...	...	ctg	...	gc.	...	..t	..t	...	..g	..a
6	..t	..c	...	..c	...	..a	.t.	..a	...	..t	..t	ctg	...	gc.	...	..t	..t	...	..g	..a
7	..t	..c	...	..c	...	..a	.t.	..a	...	..t	..t	ctg	...	gc.	...	..t	..t	...	..g	..a
8	..t	..c	...	..c	...	..a	.t.	..a	...	..t	...	ctg	...	gc.	...	..t	..t	...	..g	..a
9	..t	..c	...	..c	...	..a	.t.	..a	...	..t	...	ctg	...	gc.	...	..t	..t	...	..g	..a
10	..t	..c	...	..c	...	..a	.t.	..a	...	..t	...	-t.	..g	gc.	...	..t	..t	...	..g	..a
11	..t	..c	...	..c	...	..a	.t.	..a	...	..t	..t	ct.	...	gc.	...	..t	..t	...	..g	..a
12	..t	..c	...	..c	...	..a	.t.	..a	...	..t	..t	ct.	...	gc.	...	..t	..t	...	..g	..a

	15355							15370					15385						15400
1	ttc	cta	cat	gaa	act	gga	tca	aag	aac	cca	atg	ggc	cta	aac	tca	aat	gca	gat	aaa	atc
2	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	...	...
3	...	..t	...	...	...	..g	...	..c	...	...	..t	...	...	...	..t	...	...	..c	...	...
4	...	..t	...	...	...	..g	...	..c	...	...	..t	...	...	...	..t	...	...	..c	...	...
5	...	...	...	...	..c	..g	...	..c	...	...	..t	...	...	...	..t	...	...	..c	...	...
6	...	...	...	..g	..c	..g	...	..c	...	...	..t	...	...	...	..c	...	...	..c	..g	...
7	...	...	...	..g	..c	..g	...	..c	...	...	..t	...	...	...	..c	...	...	..c	...	...
8	...	...	...	...	..c	..g	...	..c	...	...	..t	...	...	...	..c	...	...	..c	...	...
9	...	...	...	...	..c	..g	...	..c	...	...	..t	...	...	...	..c	...	...	..c	...	...
10	...	...	...	...	..c	..g	..g	..c	...	...	..t	...	...	...	..c	...	..g	..c	...	...
11	...	...	...	...	..c	..g	..g	..c	...	...	..t	...	...	...	..t	...	..g	..c	...	...
12	...	...	...	...	..c	..g	..g	..c	...	...	..t	...	...	...	..t	...	..g	..c	...	...

	15415							15430					15445						15460
1	tcc	ttc	cac	ccc	tac	ttc	acc	tac	aaa	gat	gcc	cta	gga	ttt	gcc	atc	ctt	ctt	atg	gct
2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
3	..g	...	..t	..g	...	..t	..g	..t	...	..c	ct.	..c	..c	...	...	g.t	..c	...	..a	.gc
4	..g	...	..t	..g	...	..t	..g	..t	...	..c	ct.	..c	..c	...	...	g.t	..c	...	..a	.gc
5	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	..c	..c	g.a	..c
6	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	..c	..c	g..	..c
7	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	..c	..c	g..	..c
8	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	..c	..c	g..	.
9	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	..c	..c	g..	..c
10	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	...	..c	g..	..c
11	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	...	..c	g..	..c
12	...	...	...	..a	...	...	...	...	...	..c	ct.	..t	..c	...	...	g..	...	..c	g..	..c

	15475							15490		
1	ctt	aaa	tcc	cta	gca	ctc	ttc	tcc	ccc	aac	ct
2	..									
3	...	.cc	...	...	...	...	...	...	..g	...	.
4	...	.cc	...	...	...	...	...	...	..g	...	.
5	...	tcc	..t	...	...	...	.				
6	..c	tcc	..t	..c	...	...	...	..t	...	...	..
7	..c	tcc	..t	..c	...	...	...	..t	...	...	..
8											
9	...	tcc	..								
10	..c	tcc	..t	..c	...	...	...	c.t	...	...	..
11	..c	tcc	..t	..							
12	..c	tcc	..t	..							

[1] Block, B. 1993. Personal communication.
[2] GenBank accession no. V00662.

vided no evidence for the hybrid origin of individuals of intermediate coloration (Kijima et al., 1986).

The systematic status of geographically distant *S. japonicus* populations has been problematic because this species is characterized by considerable morphological variability. Several names have been used for the groups occurring in different areas: *S. japonicus*, western North Pacific (Houttuyn, 1782); *S. colias*, eastern Atlantic (Gmelin, 1789); *S. grex*, western North Atlantic (Mitchell, 1815); *S. diego*, eastern North Pacific (Ayres, 1857); *S. peruanus*, eastern South Pacific (Jordan and Hubbs, 1925); and *Pneumatophorus japonicus marplatensis*, western South Atlantic (López, 1955). Differences in pigmentation, tooth crenulation, scale size, and gill-raker counts on the lower first arch distinguish fish from the Pacific, western Atlantic, and eastern Atlantic. These groups were synonymized into the single species *S. japonicus* by Matsui (1967) because "[morphological] differences are smaller [between populations] than those which separate sympatric species of *Scomber* and *Rastrelliger*, and it thus seems correct to regard *S. japonicus* as a single polytypic species."

The mtDNA data did not refute the specific status of *S. japonicus* and *S. australasicus* reported by Matsui (1967). In fact, a greater divergence was found among two mtDNA lineages within *S. australasicus* than between any other pairs of samples of *S. japonicus* and *S. australasicus*. The two divergent *S. australasicus* mtDNA lineages, which differed by an average of δ=1.84% according to the restriction site data, were equally represented in both South Pacific collections. A cursory allozyme analysis of 10 loci from 10 individuals of each lineage revealed no significant allelic differences in nuclear encoded genes (Scoles, 1994). The phylogenetic pattern observed in *S. australasicus* appears to be the result of postdivergence introgressive hybridization.

A similar phylogenetic pattern has been observed in other pelagic marine fishes. In blue marlin (*Makaira nigricans*), "ubiquitous" haplotypes occurring in both the Atlantic and Pacific oceans were highly divergent from other Atlantic haplotypes, forming a "unique" clade revealed by mtDNA analysis restriction fragment analysis (n=114, δ=0.15%, Graves and McDowell, 1995) and by sequencing 612 bp of the mtDNA cytochrome *b* gene (n=26, δ=1.6%, Finnerty and Block, 1992). Similarly, sailfish (*Istiophorus platypterus*) had Pacific and Atlantic clades that differed by δ=0.27% (Graves and McDowell, 1995). The patterns in these fishes and in *S. australasicus* demonstrate that considerable mtDNA divergence can occur among haplotypes within a single sample, and suggest the limitations of basing taxonomic status only on mtDNA data.

The mtDNA data indicated the possibility of multiple taxonomic lineages within *S. japonicus*. The two major groups of *S. japonicus* (Atlantic and Pacific) are differentiated by nearly as much as are the two different lineages within *S. australasicus*. The nucleotide sequence divergence between some *S. japonicus* groups is nearly as great as between groups of *S. japonicus* and *S. australasicus* (Table 4). Unlike the two divergent *S. australasicus* lineages that were observed in single samples, the *S. japonicus* groups are geographically partitioned. The paraphyletic relationship (Figs. 3–5), geographic isolation, high level of genetic divergence (Table 4), and morphological differences among the *S. japonicus* lineages support recognition of separate species: *Scomber japonicus* Houttuyn, 1782, in the Pacific; and *Scomber colias* Gmelin, 1789, the Atlantic. This conclusion assumes that there is no significant sampling error (see Moore, 1995) and should be tested by further morphological and nuclear DNA data analyses. The Red Sea population of *Scomber*, previously considered as *S. japonicus* (Matsui, 1967), was re-examined by Baker and Collette (1998), who have assigned the Red Sea-northern Indian Ocean population to *Scomber australasicus* on the basis of morphological characters.

Phylogeographic patterns and their origins

The distribution of haplotypes between eastern and western Atlantic samples of *S. scombrus* indicated the populations do not share a common gene pool. The close relationship among haplotypes in these samples (Fig. 2) contrasts with the finding of two divergent mtDNA matrilines (δ=3.7%) in samples of capelin, *Mallotus villosus*, from eastern and western regions of the North Atlantic (Dodson et al., 1991). An intermediate level of divergence to that of *S. scombrus* and of capelin was observed between samples of bluefish (*Pomatomus saltatrix*, δ=0.26%, 1 fixed difference) from the eastern and western Atlantic Ocean (Goodbred and Graves, 1996). The lower divergence in *S. scombrus* may be the result of more recent isolation or greater vagility.

Patterns of divergence in *S. japonicus* and other species in the Atlantic Ocean appear to be highly influenced by species' vagility and warm water in the tropics. Haplotype distributions were significantly different between *S. japonicus* samples from the western Atlantic Ocean (J-FLA and J-ARG), and unique haplotypes occurred within each at relatively high frequencies (10–20%). Similary, a population-genetic study of the shortfin mako shark demonstrated significantly different mtDNA haplotype frequencies between samples from the western North and South Atlantic Ocean (δ=0.13%, P<0.001) (Heist

et al., 1996). Bluefish sampled from Brazil and the eastern U.S. coast were even more divergent (δ=1.48%) (Goodbred and Graves, 1996). But in the eastern Atlantic, no significant differentiation was seen among *S. japonicus* samples from the eastern Mediterranean Sea (J-ISR), Ivory Coast (J-IVC), and South Africa (J-SAF). Additionally, although no haplotypes were shared between bluefish samples from the Mediterranean Sea and South Africa (δ=0.35%), haplotypes in the two samples were closely related (Goodbred and Graves, 1996). In contrast, the less mobile gray mullet, *Mugil cephalis*, sampled from the Mediterranean Sea, and South Africa were highly divergent (δ=2.92) (Crosetti et al., 1994). These data suggest that fewer barriers to gene flow occur between northern and southern regions of the eastern Atlantic versus those of the western Atlantic. The tropical water of the east Atlantic occupies the smallest area of all the tropical regions, and temperate species are suspected of crossing this region by moving under the warm water mass, thereby achieving an antitropical distribution (Briggs, 1974).

It is possible that *S. japonicus* larval or adult exchange occurs between Gulf of Guinea and the northernmost extension of the southwest Atlantic *S. japonicus* population assisted by trans-Atlantic equatorial currents. These locations are separated by the most narrow region of the Atlantic Ocean. Haplotype frequencies were significantly different among all samples across the Atlantic Ocean except in one pairwise comparison of J-ARG and J-IVC. Genetic similarity between these samples may reflect recent isolation or contemporary gene flow. Eastern and western Atlantic populations of *S. japonicus* are, however, morphologically differentiated on the basis of nonoverlapping gill-raker counts (Starks, 1921; Matsui, 1967).

The sample of *S. australasicus* from the Red Sea (A-RED) had the highest estimated haplotypic diversity. No haplotypes were shared between the samples of *S. japonicus* from the Atlantic Ocean and the Red Sea, suggesting no gene flow through the Suez Canal. Fifty-two fish species are known to have made the passage, all but one from the Red Sea to the Mediterranean Sea (Golani, 1993). *Rastrelliger kanagurta*, a close relative of *Scomber*, is among those known to have invaded the Mediterranean Sea from the Red Sea (Collette, 1970). The samples of *S. japonicus* from the eastern Mediterranean Sea (J-ISR) and *S. australasicus* from the Red Sea were divergent by δ=0.72%, one fixed restriction site difference, and an additional site that was nearly fixed. These findings do not exclude the possibility of movement through the Suez Canal, which may be revealed by larger sample sizes.

Fishery data indicate that *S. japonicus* in the region of Japan and Taiwan represent separate populations with unique spawning grounds and larval retention areas (Sato, 1990). Although haplotypes in the sample from the eastern Pacific (J-CAL) were not found in other *S. japonicus* samples, haplotypes in *S. japonicus* samples from the northwest region of the Pacific Ocean (J-JPN and J-TWN) were shared at similar frequencies. The data of our study are consistent with exchange among the two western Pacific populations. Whether exchange is the result of larval drift or adult movement is indeterminable; however, movement of adult *S. australasicus* is known to occur between these regions (Chang and Wu, 1977). Greater trans-Pacific divergence than that observed in *S. japonicus* was seen in gray mullet sampled from Hawaii and the Galapagos Islands (δ=1.4%) (Crosetti et al., 1994), suggesting these population were isolated for a considerably longer period than mackerel.

The level of nucleotide sequence divergence between Atlantic and Pacific samples of *S. japonicus* was lower than expected. It has been suggested that in mammals, mitochondrial DNA evolves at rate of 2% nucleotide sequence divergence per million years (Brown et al., 1979). When this rate of divergence is applied to the 1.4% net nucleotide sequence divergence observed between Atlantic and Pacific *S. japonicus*, it appears that the two populations were isolated about 700,000 years. Such estimates of divergence are approximate at best, but if this rate of divergence is valid in *Scomber japonicus*, the observed nucleotide sequence divergence is lower than would have resulted if these populations were isolated by the uplift of the Isthmus of Panamá, 3.1–3.6 million years ago (Keigwin, 1978). Gene flow may have occurred between the two regions since the uplift of Panamá, during times of warmer climates (Avise, 1992).

Samples of *S. australasicus* between New Zealand and Australia, regions separated by nearly 2000 km of deep ocean, were genetically homogeneous. Perhaps there is a high potential for genetic exchange in *S. australasicus*, which was also indicated by comparison of *S. australasicus* samples from the northern Pacific. Although a test of homogeneity between A-JPN and A-MEX was marginally significant (P=0.013), the mean nucleotide sequence divergence was only 0.02%, which contrasted with the 0.30% divergence and fixed restriction site difference between samples of *S. japonicus* from the eastern and western Pacific Ocean.

The lowest genotypic and nucleotide sequence diversity in the study occurred in a sample from the Revillagigedo Islands (A-MEX) and may indicate the origin of this population by means of a founder event

from a source population in the northwest Pacific region or Hawaii. Colonization of the Revillagigedo Islands may have occurred through the migration of adults. Gene flow between other isolated populations (J-IVC and J-ARG; A-NZL and A-AUS) was also suggested by the mtDNA data and may be facilitated by the high vagility of species of *Scomber*.

Despite the genetic similarity observed among widely separated samples of *S. australasicus*, exchange in the north-south direction is restricted. Haplotypes of the unique New Zealand-Australia lineage were not observed in samples from Japan or Mexico. These data suggest that the equatorial region of the Pacific is a barrier to gene flow in *S. australasicus*, and together with the significant difference observed between western Atlantic *S. japonicus* samples, strongly emphasizes the impact of physical oceanographic conditions in shaping phylogeographic patterns. *Scomber japonicus* from the eastern North and South Pacific were also differentiated by allozyme analysis which revealed three differentiated loci of fourteen that were variable (Nei's D=0.033, F_{st}=0.20) (Stepien and Rosenblatt, 1996). These observations support the hypothesis that physical oceanographic characteristics of equatorial regions may limit gene flow in species of *Scomber*, and possibly a variety of other temperate pelagic species.

These data demonstrate that levels of heterogeneity reflect species vagilities and are shaped by physical oceanographic processes. Other studies have demonstrated that population structure in several species is generally lower between populations separated by vast ocean basins than between those separated by land masses, in some cases suggesting continued gene flow (Vawter et al., 1980; Rosenblatt and Waples, 1986; Shulman and Bermingham, 1995). But not all genetic data support a simple relationship between the degree of geographic barriers, dispersal, and genetic relatedness. In one study, five species with various life history features that limit dispersal showed no intraspecific differentiation, whereas one, *Gnatholepis thompsoni*, which had nonpelagic eggs and a long planktonic life, showed population structure across the Caribbean Sea (Shulman and Bermingham, 1995). Sinclair (1988) reviewed the effects of oceanographic characteristics on population structure and concluded that it is defined at the planktonic phase of the life history, rather than at the juvenile or adult phases, and is largely regulated by the physical environment. However, Thresher and Brothers (1985) were unable to relate larval stage duration or adult size to dispersal in several angelfish species (Pomacanthidae) and suggested that geological features are better predictors of dispersal. Clearly, the processes by which population structure emerges in marine fishes are complex. Our data support the hypothesis that oceanographic characteristics are significant in limiting dispersal in *Scomber*, especially in some equatorial regions. The exchange of adult individuals among populations appears to have a significant effect on limiting the divergence between some populations, possibly reflecting adaptations in *Scomber* for survival in the pelagic environment.

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