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Global phylogeography of yellowfin tuna, *Thunnus albacares*, and mackerels of the genus *Scomber*

Scoles, Daniel R., Ph.D.

The College of William and Mary, 1994

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Ann Arbor, MI 48106

Global Phylogeography of Yellowfin Tuna, *Thunnus albacares*, and Mackerels of the Genus *Scomber*

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

---

by

Daniel R. Scoles

1994

APPROVAL SHEET

This dissertation is submitted in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Daniel R. Scoles

Approved: July 1994

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In memory of my mother

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I am indebted to Kurt Schaefer for his assistance in arranging collections of specimens of yellowfin tuna and mackerel. I also wish to thank the following who assisted with collections of mackerels: Juan M. Díaz de Astarloa, Daniel Golani, Clive Roberts, J. Brian Jones, Julian Pepperell, Kent Carpenter, Emile G. Marchal, Lounês Chikhi, P. Alexander Hulley, Liz Hoenson, Hin-Kiu Mok, Yu-Yun Chen, Yoshiro Watanabe, Sachiko Tsuji, Chris Martin, Eugene L. Nakamura, Barbara J. Palko, Ed Everett, Mitsu Yesaki, G. A. Potts, A. Frettsome. I thank Ray Webster and Peter Burn of Air New Zealand for volunteering to transport specimens from New Zealand. Regional literature was made available for my use by Antony Lewis and Ziro Suzuki.

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ABSTRACT

Intraspecific genetic relationships within yellowfin tuna, *Thunnus albacares*, and three mackerels of the genus *Scomber* were studied by restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA). The comparison of these scombrids, with different larval distributions, adult distributions, and vagilities, served to investigate the hypothesis that population structure in marine fishes results from geographic and physical oceanographic processes that limit dispersal of early life history stages.

Samples of 20 yellowfin tuna were examined from each of five Pacific locations and one Atlantic location. MtDNA analysis with 12 informative restriction endonucleases demonstrated considerable genetic variation, as evidenced by overall nucleon diversity of 0.84 and mean nucleotide sequence diversity of 0.31%. Estimates of within-sample variation were consistent across all six locations. Common genotypes occurred with similar frequencies in all samples, and with one exception, all genotypes that were represented by more than one individual occurred at more than one location. The null hypothesis that the sampled populations of yellowfin tuna share a common gene pool was not rejected.

In contrast, analysis of species of *Scomber* revealed considerable intraspecific differentiation. A total of 15 samples averaging 19 individuals each of *Scomber japonicus*, *S. australasicus*, and *S. scombrus* were collected from geographically isolated populations throughout the ranges of each species. Genotypic diversities ranged from 0.28 to 0.95, and nucleotide sequence diversities from 0.13 to 0.76%. East and west Atlantic populations of *S. scombrus* exhibited significant differentiation, but no fixed restriction site differences. This species differed by 11.9% nucleotide sequence divergence from the other two species. In *S. japonicus*, fixed restriction site differences were revealed among Pacific samples, but not among Atlantic samples; although significant heterogeneity occurred within the Atlantic. In *S. australasicus*, North and South Pacific samples were highly differentiated. One of two divergent mtDNA matrilines observed in this species was restricted to southern samples.

The study demonstrated that population structure is greater in the species of *Scomber* than in yellowfin tuna. It further revealed that adult dispersal, in addition to geographic and physical oceanographic processes that control dispersal of early life history stages, are of significant importance in shaping population structure in scombrids.

Global Phylogeography of Yellowfin Tuna, *Thunnus albacares*, and Mackerels of the Genus *Scomber*

INTRODUCTION

The pelagic environment presents few physical barriers that limit passive drift of larvae or movements of fishes. Consequently, gene flow in marine fishes can be extensive, resulting in limited population-genetic structure. By comparison, freshwater fishes typically exhibit considerable population structure, as gene flow among drainages is often restricted by geographic barriers, promoting genetic divergence (Gyllensten, 1985; Bermingham and Avise, 1986). However, marine fishes do exhibit varying levels of heterogeneity which possibly reflect differences in spawning habits, demersal egg deposition, planktonic larval duration, adult vagility, and physical oceanographic characteristics that may limit dispersal (Rosenblatt, 1963; Hanson, 1978; Bell et al., 1982; Sinclair, 1988).

Sinclair (1988) noted a relationship between population structure in marine fishes and physical geography and oceanography that control retention of early life history stages. His observations led to the formulation of the member/vagrant hypothesis which holds that population structure is a function of the number of areas in which a species' life cycle is capable of closure, and physical oceanographic factors that affect losses of individuals from the population. Sinclair (1988) supported the hypothesis by descriptions of marine fishes with high and low population structures. Species with low population structure often possess long-lived pelagic larvae capable of extensive dispersal. An

example of this is the leptocephalus larvae of the catadromous American and European eels (*Anguilla rostrata* and *Anguilla anguilla*, respectively), which passively drift from the Sargasso Sea to adult freshwater habitats. Analysis of the population-genetic structure of these species has indicated that each species is not statistically different from a panmictic population (Avise et al., 1986). Species with separate larval retention areas may be characterized by intraspecific differentiation, exemplified by genetic analysis of the black sea bass, *Centropristis striata*. Mitochondrial DNA (mtDNA) analysis revealed 5 fixed restriction site differences between black sea bass samples from the Gulf of Mexico and the northwest Atlantic, where separate spawning populations occur (Bowen and Avise, 1990).

In general, genetic analyses of marine fishes have revealed levels of divergence consistent with Sinclair's member/vagrant hypothesis, although there are numerous exceptions. Winans (1980) found very little allozymic differentiation among populations of milkfish (*Chanos chanos*) sampled from throughout the central and western Pacific, which he attributed to gene flow mediated by larval drift among populations. Allozyme analysis of 12 species of shorefishes from eastern and western Pacific locales separated by 5,000 km revealed low levels of differentiation (Rosenblatt and Waples, 1986). Little genetic divergence has also been demonstrated among distant populations of demersally spawning fishes. An allozyme study of damselfish (*Stegastes fasciolatus*), with demersal attached eggs and short-lived pelagic larvae, sampled from throughout the Hawaiian Archipelago, revealed no intraspecific differentiation (Shaklee, 1984). Similarly, Lacson (1992) demonstrated little allozymic differentiation within five demersally spawning

damselfish species (*Chromis cyanea*, *Stegastes partitus*, *S. planifrons*, *S. leucostictus*, and *S. dorsopunicans*) sampled from isolated Caribbean reefs. Large, pelagic species have also been characterized with little intraspecific differentiation. Analysis of mitochondrial DNA of skipjack tuna (*Katsuwonus pelamis*) and albacore (*Thunnus alalunga*) samples from the Atlantic and Pacific oceans revealed no evidence for differentiation, possibly a result of the high vagility and continuous distributions of these species (Graves et al., 1984; Graves and Dizon, 1989).

In contrast, population structure has been demonstrated within other marine fish species with broad geographic distributions. In the anemonefish (*Amphiprion clarkii*), a species with low mobility, demersal spawning, and limited pelagic larval duration, allozyme analysis revealed considerable genetic divergence among populations separated by deep ocean basins (Bell et al., 1982). Four fixed allelic differences were demonstrated between two species of damselfishes (*Chrysiptera cyanea* and *Pomacentrus coelestis*) from Palau and Okinawa (Lacson, 1994). Three fixed mtDNA restriction site differences were observed between bluefish (*Pomatomus saltatrix*) populations sampled from the east coasts of North America and Australia (Graves et al., 1992b). Analysis of mtDNA of the cosmopolitan gray mullet (*Mugil cephalis*) revealed unique genotypes from each of 7 sampling locations from around the world (Crosetti et al., 1994), indicating genetic isolation among populations.

The above examples illustrate that intraspecific differentiation is not always found in species with life history characteristics that limit larval dispersal, and suggest that other factors may also influence population structure in broadly distributed marine fishes.

In effort to elucidate such factors that shape population structure, the present study provides an analysis of the global population genetics of two groups of vagile, pelagic, marine fishes: yellowfin tuna, *Thunnus albacares*, and three species of mackerel of the genus *Scomber*.

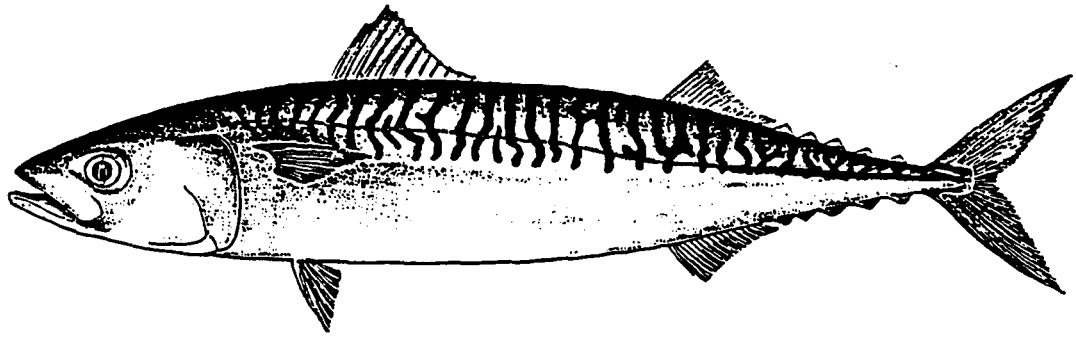
Choice of species

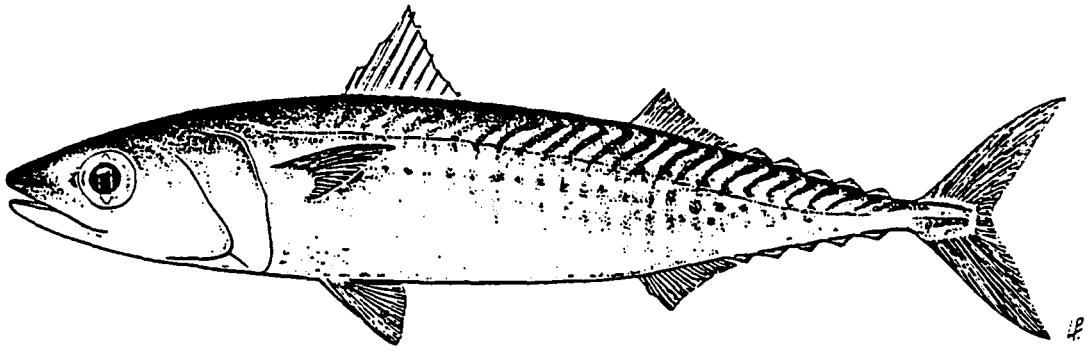
To obtain insights into the factors that control population structure in pelagic marine fishes, this study focuses on yellowfin tuna and mackerels of the genus *Scomber*. These fishes probably represent the extremes of the spectrum of low to high population structure within the family Scombridae. Each of the three species of *Scomber* (Fig. 1) has a unique distribution: Atlantic mackerel, *Scomber scombrus*, occurs only in the North Atlantic, spotted chub mackerel, *S. australasicus*, occurs only in the Pacific, and chub mackerel, *S. japonicus*, is cosmopolitan. Species of *Scomber* are neritic and distributed among disjunct populations (Figs. 2-4). In contrast to mackerels, yellowfin tuna (Fig. 5) is oceanic and is continuously distributed circumtropically (Fig. 6).

Both yellowfin tuna and mackerels of the genus *Scomber* exhibit considerable morphological variation. So much in fact, that early studies of both yellowfin tuna and *S. japonicus* recognized several species that were later synonymized (Gibbs and Collette, 1967; Matsui, 1967).

Yellowfin tuna and mackerels of the genus *Scomber* differ in vagility and the distribution of spawning areas. The yellowfin tuna is much larger and more vagile than

Figure 1. Illustration of mackerels of the genus *Scomber*. Top: Atlantic mackerel, *Scomber scombrus*. Center: Spotted chub mackerel, *Scomber japonicus*. Bottom: Chub mackerel, *Scomber japonicus*. From Collette and Nauen (1983).





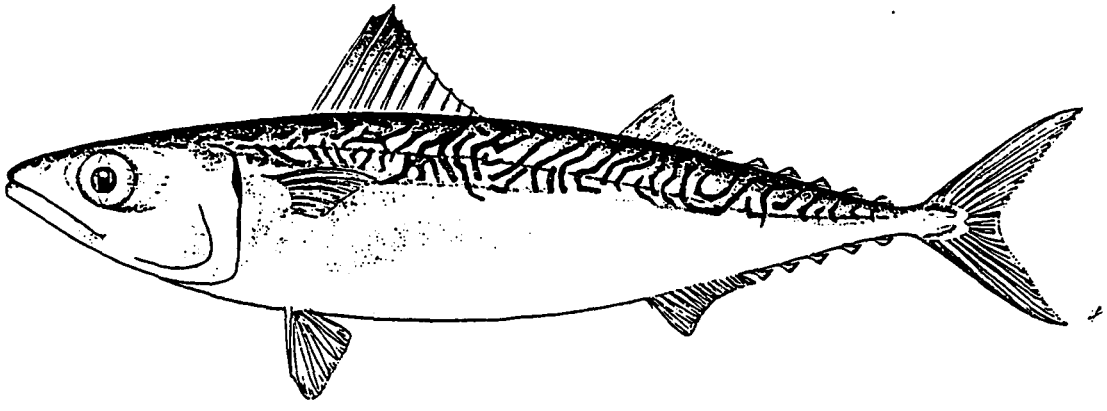


Figure 2. Sampling and distribution of Atlantic mackerel, *Scomber scombrus*, according to Collette and Nauen (1983).

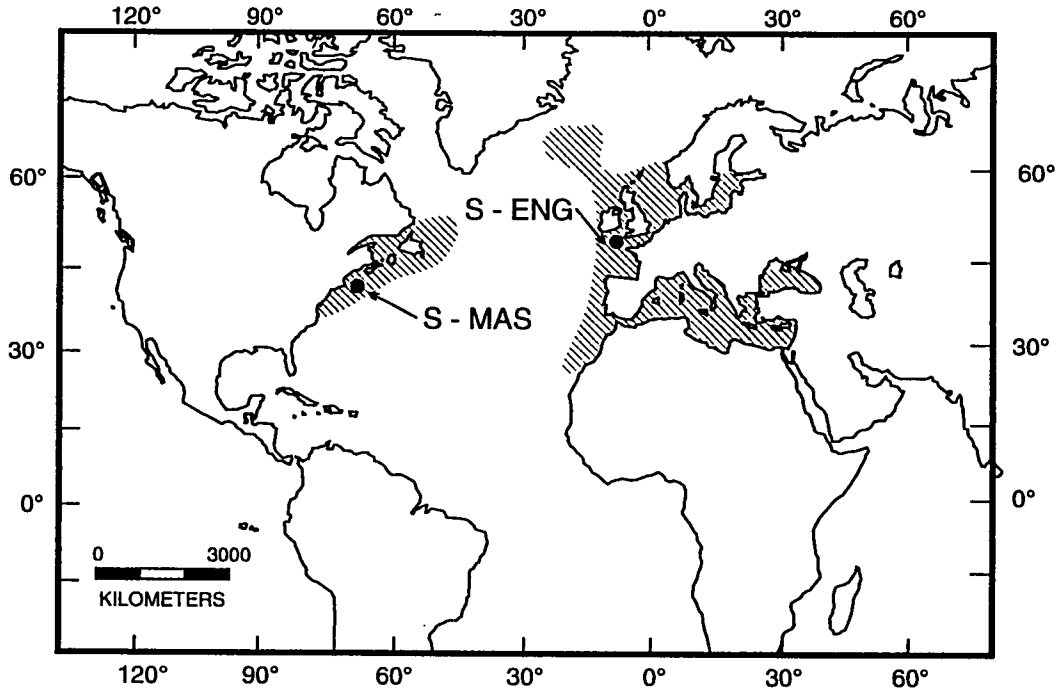

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Figure 3. Sampling and distribution of spotted chub mackerel, *Scomber australasicus*, according to Collette and Nauen (1983).

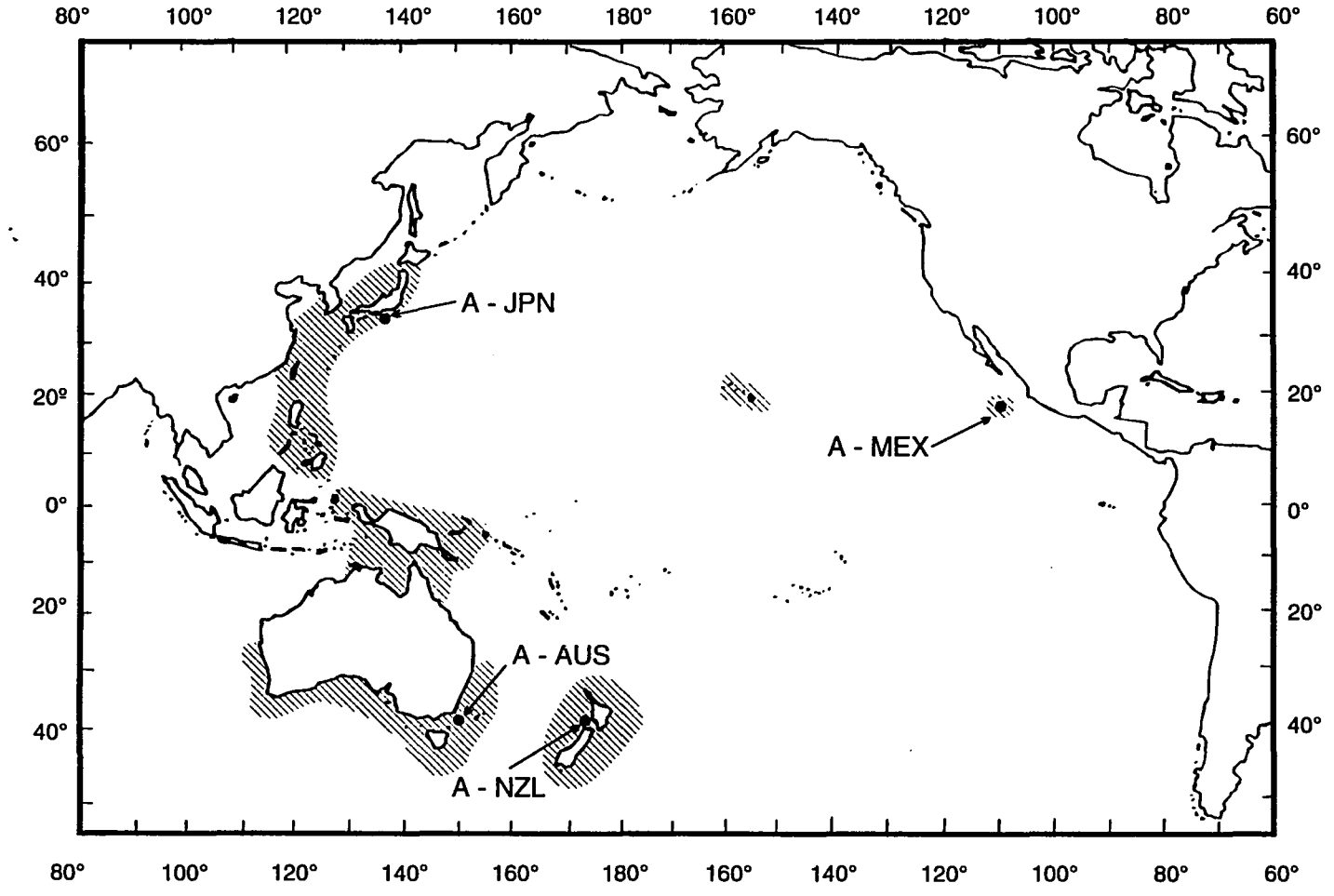
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Figure 4. Sampling and distribution of chub mackerel, *Scomber japonicus*, according to Collette and Nauen (1983), with distributions off Brazil and Namibia redrawn according to Perrotta and Aubone (1991) and Zenken and Lobov (1989), respectively.

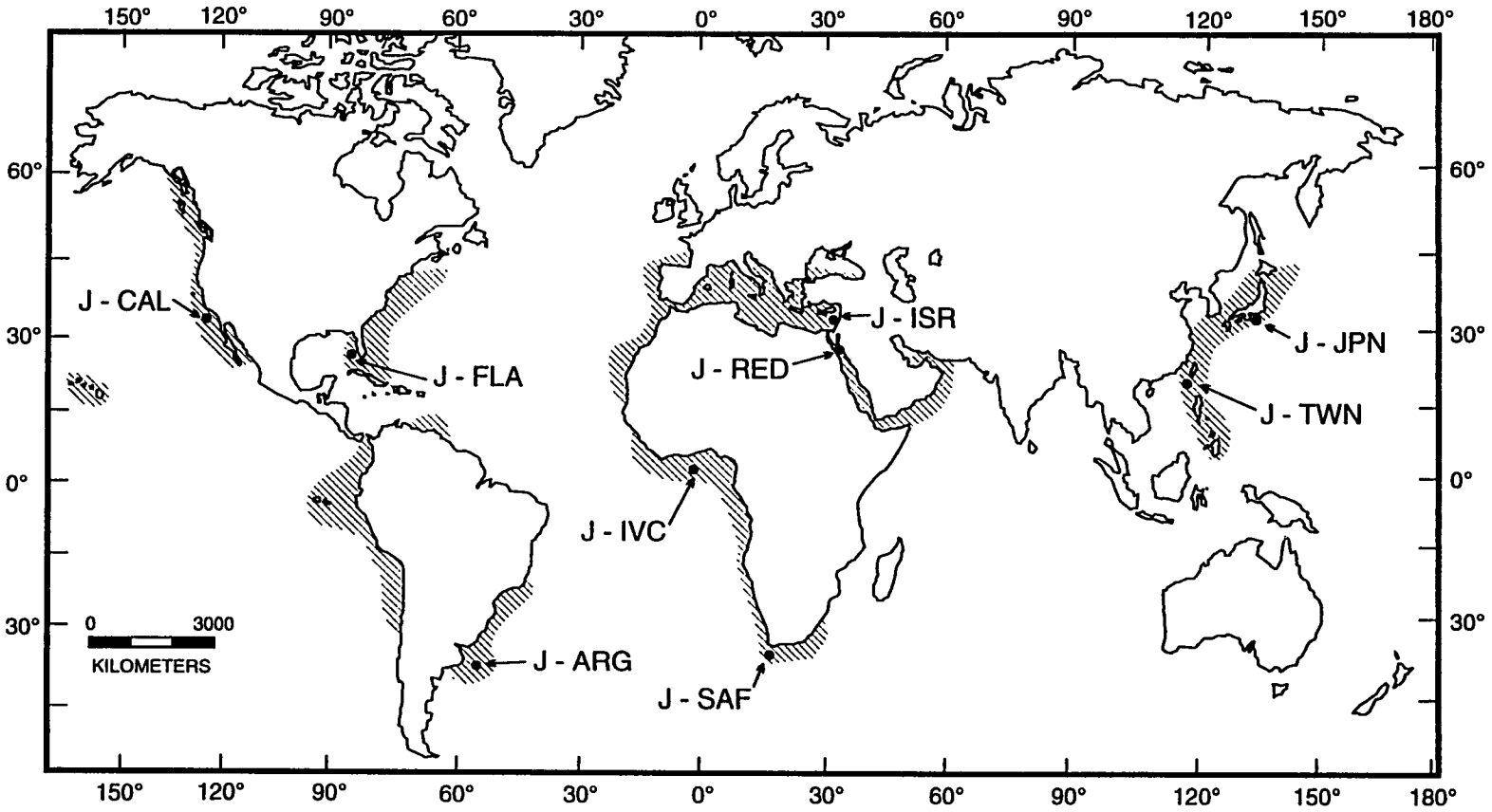
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Figure 5. Illustration of yellowfin tuna, *Thunnus albacares*. From Collette and Nauen (1983).

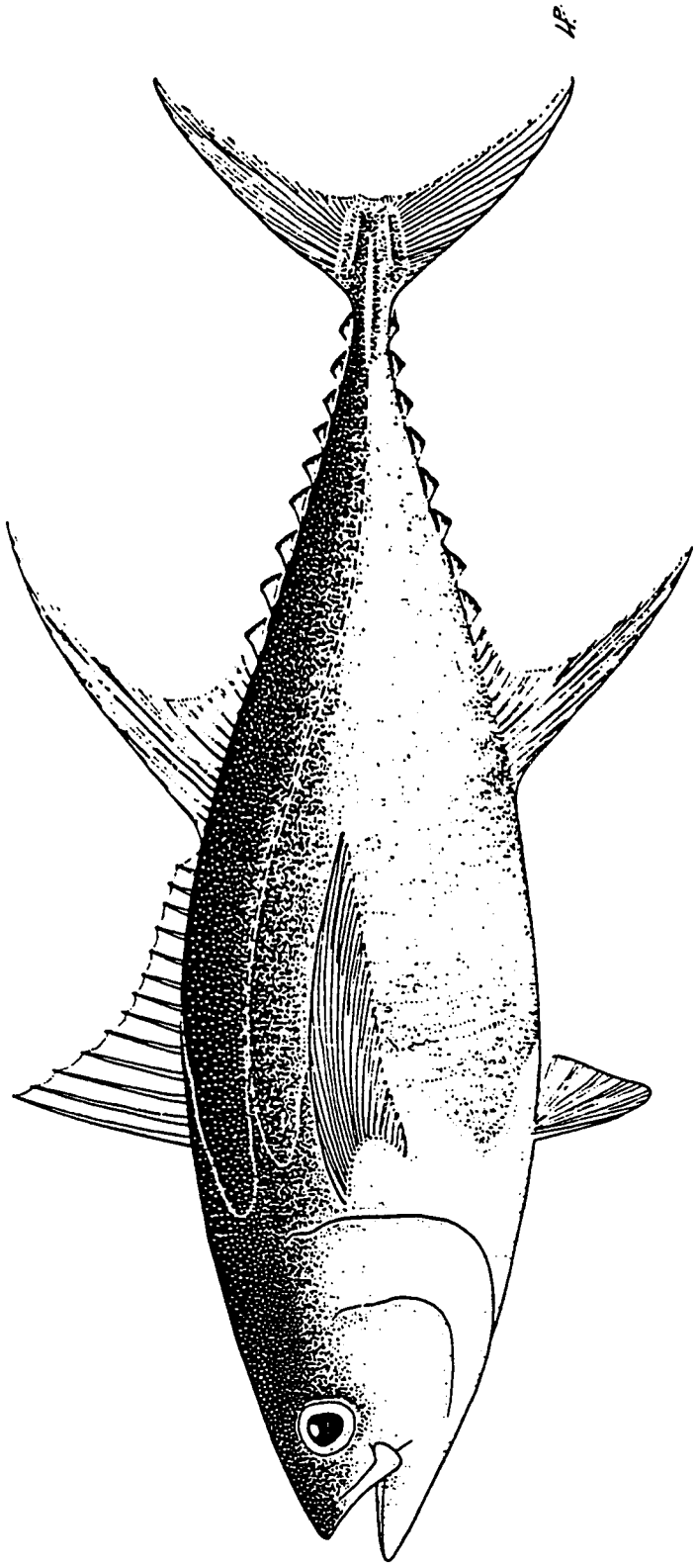
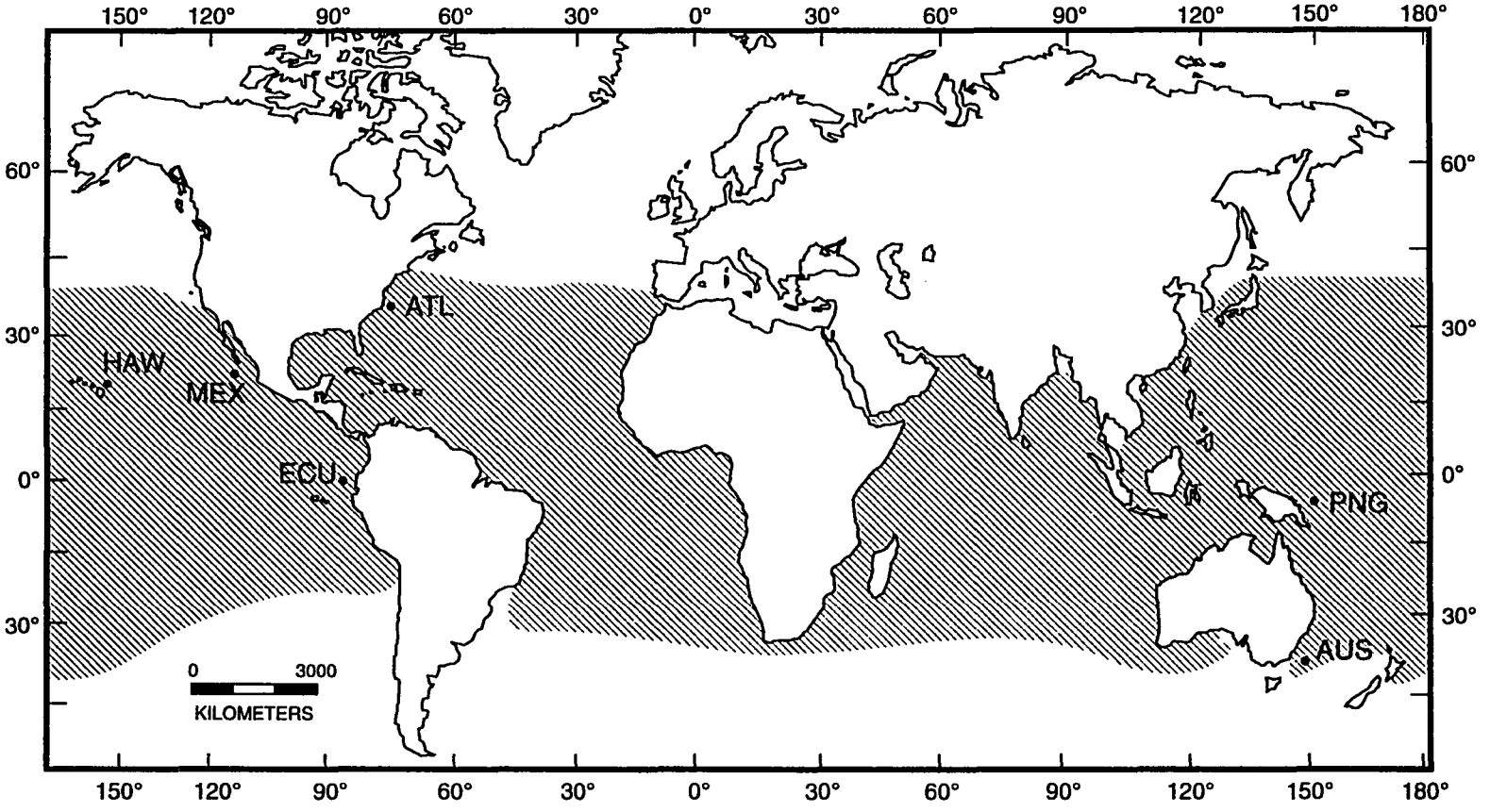


Figure 6. Sampling and distribution of yellowfin tuna, *Thunnus albacares*, according to Collette and Nauen (1983).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the mackerels, although it exhibits lower mobility relative to most other tunas (Miyabe and Bayliff, 1987). Tagging studies have shown that yellowfin tuna are capable of moving great distances (>2,000 km) between regions in the Pacific (Bayliff, 1984; Itano and Williams, 1990), while the movement of species of *Scomber* is limited to within major geographic regions of occurrence (Chang and Wu, 1977; Sette, 1950; Hamre, 1980; Sato, 1990). Spawning areas of yellowfin tuna are distributed circumtropically and continuously throughout the world's equatorial regions (Nishikawa et al., 1985), whereas those of *S. japonicus* and *S. scombrus* are coastal (Hunter and Kimbrell, 1980; Ware and Lambert, 1985; Sato, 1990).

Choice of method

The selection of the genetic character for analysis depends on the problem under investigation. A character that is rapidly evolving is best suited for the study of closely related groups, and is essential for intraspecific analyses, while comparisons across distantly related taxa are best accomplished by analysis of a conserved character. Regions of the DNA that encode proteins are generally more conserved than non-coding regions that are not as likely to be affected by selection. Of the structural genes, those that code for proteins required for enzymatic or structural functions, cytochromes, hemoglobins, and ribosomes are more conserved than dehydrogenases and kinases (Futuyma, 1986). Structural genes are collectively more conserved than regulatory elements that control their expression (Suzuki et al., 1981).

Descriptions of genetic variability in fishes have most commonly been accomplished

by allozyme analysis. The advantages of this technique is that a considerable region of the genome can be screened, many individuals can be assayed rapidly, and at a relatively low cost (Ryman and Utter, 1986). However, direct DNA analysis may be more informative than allozyme analysis, because the allozyme technique depends upon amino acid differences that affect electrostatic charges or protein conformations that alter electrophoretic mobility. Thus "silent" mutations at the DNA level which do not result in amino acid substitutions are not seen in the results of allozyme electrophoresis.

Over the past 10 years there has been increasing use of RFLP analysis of mtDNA to examine population structure of marine fishes. MtDNA is useful in population-genetic studies because it evolves rapidly relative to nuclear DNA (Brown et al., 1979), and is highly polymorphic in animal populations (Avise and Lansman, 1983). MtDNA is also inherited clonally (maternally), which, because it is haploid, reduces the effective population size 4 fold, thus evolutionary changes due to genetic drift accumulate faster in mtDNA than in nuclear DNA (Birky et al., 1983). An additional advantage to RFLP analysis of mtDNA is that the whole molecule is sampled, including regions of varying degrees of conservatism. This is desired as the evolution of a single gene may not accurately reflect the evolution of the entire genome. Analysis of both nuclear DNA and mtDNA by direct analysis of RFLPs have at times made it possible to identify significant intraspecific differentiation where allozyme analysis has not (Saunders et al., 1986; Reeb and Avise, 1989; Karl and Avise, 1992).

Other methods are available that allow direct analysis of DNA. Many of these are based on the polymerase chain reaction (PCR), which is used for the amplification of a

region of DNA of interest. The amplified region can be studied by sequence analysis, or by restriction fragment length polymorphism (RFLP) analysis. The PCR method allows the amplification of a rapidly evolving or conserved region.

The technique of RFLP analysis of mtDNA was employed to survey population structure within yellowfin tuna and mackerels. Phylogenetic comparisons among species of *Scomber* were accomplished by analysis of a conserved region of the mitochondrial DNA, cytochrome *b*, by PCR amplification and direct sequencing. This technique was selected as it allows ease of determination of homologous characters among taxa, a process in RFLP analysis of mtDNA that was complicated by a high level of genetic divergence observed among the species.

Population-Genetic Structure of Yellowfin Tuna, *Thunnus Albacares*

Introduction

Yellowfin tuna (*Thunnus albacares*) occur in the tropical and subtropical oceans and support major commercial fisheries throughout their range (Collette and Nauen, 1983). The economic importance of this species is indicated by high annual catches which have increased from 596,764 mt in 1981 to 986,529 mt in 1990, of which 66 to 69% were from the Pacific Ocean (FAO, 1992). Recently, purse-seine and longline fisheries in the western Pacific provided a major share of yellowfin tuna landings, with a catch of 342,921 mt in 1990 (Lawson, 1991). In the eastern Pacific (east of 130°W) record landings near 270,000 mt occurred in each of the past 3 years (Anonymous, 1992).

A thorough understanding of yellowfin tuna population structure is necessary for the effective management of this economically important, marine resource. A variety of studies, including tagging, morphometric, fishery statistic and genetic analyses, have been used to infer population structure. However, the proposed population structures differed.

Tagging studies have indicated that movements of yellowfin tuna in the Pacific Ocean tend to be geographically restricted. Fink and Bayliff (1970) analyzed tag return data in the eastern Pacific and proposed a northern and southern group of fish with some exchange between groups. There was very limited westward movement of tagged fish reported in the study; however, as the eastern Pacific fishery expanded westward in

subsequent years, several returns were obtained from farther offshore indicating possible mixing between eastern and central Pacific fish (Bayliff, 1984). In the western Pacific (120°E to about 180°) tagging studies showed that most individuals remained within the western Pacific region and did not make extensive movements (Itano and Williams, 1992; Lewis, 1992). Although the majority of recaptured yellowfin tuna in all studies showed limited movement, some returns were obtained which demonstrated the potential for fish to move large distances and between regions (Fink and Bayliff, 1970; Bayliff, 1984; Itano and Williams, 1992).

Population structure was indicated by investigations of both meristic and morphometric characters which revealed significant differentiation among yellowfin tuna from the eastern, central and western Pacific regions (Schaefer, 1955; Kurogane and Hiyama, 1957), as well as clinal character variation across the equatorial Pacific (Royce, 1964). Further investigation using discriminant function analysis of morphometric variables suggested mixing occurs between morphologically differentiated northern and southern yellowfin tuna of the eastern Pacific (Schaefer, 1989), as well as across the Pacific (Schaefer, 1991, 1992).

Analysis of fishery data also suggests population structuring of yellowfin tuna within the Pacific. Kamimura and Honma (1963) provided evidence for two or more semi-independent subpopulations based on size composition and catch data of equatorial Pacific yellowfin tuna from longline landings. Suzuki et al. (1978) examined longline and purse-seine length composition data and suggested the existence of semi-independent eastern, central and western Pacific subpopulations. Additionally, homogeneity within the

western Pacific was indicated by the widespread distribution of fish contaminated by radioactivity resulting from the 1954 U.S. nuclear tests at Bikini Atoll (Suzuki et al., 1978).

While the results of several analyses suggest yellowfin tuna exhibit population structure within the Pacific Ocean, genetic analyses have revealed no significant genetic differentiation. Suzuki (1962) reported that the blood agglutinogen Tg2 occurs in similar frequencies in samples from the Indian Ocean and the eastern Pacific. Additionally, allozyme analysis did not reveal genetic differentiation between samples of yellowfin tuna collected off Hawaii (n=529) and Baja California (n=207) at the polymorphic serum esterase locus, although overall variation was low (Fujino, 1970). Preliminary evidence for frequency differences occurring at two other loci (phosphoglucose isomerase and transferrin A) was reported for both within and between samples of Atlantic and Pacific yellowfin tuna (Anonymous, 1977, 1978). However further analysis has not been conducted to assess yellowfin tuna population structure.

Our understanding of the population structure of yellowfin tuna in the Pacific Ocean remains problematic. Much evidence is available which suggests that population structure exists, yet genetic analyses did not reveal differentiation among samples collected from distant locations. To further examine the genetic basis of the population structure of Pacific yellowfin tuna, restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) was employed. Because mtDNA evolves rapidly (Moritz et al., 1987; Brown et al., 1979) and displays considerable polymorphism within animal populations (Avise and Lansman, 1983), mtDNA analyses have been useful

in revealing population structure within marine fishes (Ovenden, 1990). Using this technique, considerable genetic variability within yellowfin tuna was demonstrated, but the null hypothesis that samples share a common gene pool was not rejected.

Materials and Methods

Hearts were taken from 50 yellowfin tuna at each of five Pacific locations and one Atlantic location (Fig. 6); however only 20 specimens per location were analyzed. Samples from the Pacific were collected during 1990 at Manta, Ecuador (ECU); Revillagigedo Islands, Mexico (MEX); Oahu, Hawaii (HAW); Manus Island, Papua New Guinea (PNG); and New South Wales, Australia (AUS). The sample from the Atlantic was collected during 1991 at Hatteras, North Carolina (ATL). Hearts were dissected within 12 hours of capture and placed on crushed ice. Hearts from fish collected in the Pacific were frozen at -20°C and shipped to the Inter-American Tropical Tuna Commission, La Jolla, CA, where they were stored at -20°C for more than 1 year before shipment to the laboratory. Hearts from fish collected in the Atlantic were transported on wet ice and frozen at -70°C within 4 hours of dissection.

MtDNA was purified from 3g of heart tissue from Atlantic specimens following the CsCl-ethidium bromide gradient centrifugation protocol of Lansman et al. (1981). MtDNA yields averaged about 350ng of supercoiled mtDNA per g of heart. Aliquots of mtDNA were digested with the following 12 informative restriction endonucleases (Stratagene and Bethesda Research Laboratories) according to the manufacturers' instructions: *Apa*I, *Ava*I, *Ban*I, *Bcl*I, *Bgl*I, *Dra*I, *Eco*RI, *Hin*dIII, *Nci*I, *Pst*I, *Pvu*II and

*Xho*I. Restriction fragments were end-labeled with a mixture of all four α-^{35}S-dNTPs using the Klenow fragment, electrophoresed at 2 volts/cm overnight in 1% agarose gels, and visualized by autoradiography (Sambrook et al., 1989).

Yields of supercoiled mtDNA from Pacific specimens were low, possibly due to sub-optimal storage conditions. For these specimens, mtDNA-enriched genomic DNA was isolated from 4 to 6g of heart tissue following the protocol of Chapman and Powers (1984), modified by the omission of sucrose step gradients and the use of 1.5% sodium dodecyl sulfate for mitochondrial lysis. Following restriction enzyme digestion and horizontal agarose gel electrophoresis, DNA fragments were transferred to nylon membranes by Southern transfer (Sambrook et al., 1989) and immobilized by long-wave UV irradiation. Prehybridization was conducted for 2h at 42°C in 50% formamide, 5x SSC, 5x Dendhardt's solution, 0.025mM $NaPO_4$, Ph 6.5, and 100μg/ml heat denatured calf thymus DNA. Probe DNA (mtDNA purified from extra specimens from the Atlantic) was nick-translated to incorporate biotin-7-dATP, and separated from unincorporated nucleotides by size exclusion chromatography. Probe was added to prehybridization solution at a concentration of 1 μg/200 cm^2 blot and allowed to hybridize overnight at 42°C. Following post-hybridization washes (Sambrook et al., 1989), mtDNA fragments were visualized with the BRL BluGene Non-Radioactive Nucleic Acid Detection Kit.

A 12-letter composite mtDNA genotype, indicating the fragment pattern for each restriction enzyme, was developed for each individual. Estimates of nucleon diversity (h) for each sample and for the pooled samples were computed following Nei (1987).

Nucleotide sequence divergences (d) among haplotypes were estimated by following Nei and Li (1979), with weighting based on the proportion of fragments produced by each enzyme class (Nei and Tajima, 1983), using the statistical package REAP (McElroy et al., 1992). Estimates of nucleotide sequence diversities (π; Nei and Tajima, 1981), and corrected nucleotide sequence divergence among samples (δ; Nei and Li, 1979) were calculated using REAP. Nucleotide sequence divergences were clustered by the unweighted pair-group method with arithmetic means (UPGMA) using the average linkage algorithm of the SPSS-X statistical package. Values of G_{st}, a measure of heterogeneity between samples, were estimated from sample genotype frequencies (Nei 1987), and values of N_em, the absolute number of migrants between samples, were determined from the relation $N_em = (1/G_{st} - 1)/2$ (Birky et al., 1983; Nei, 1987). Chi-square analysis was conducted using the Monte-Carlo method of Roff and Bentzen (1989) with 1000 randomizations of the data to evaluate heterogeneity of genotype frequencies among samples without combining rare genotypes.

Results

Analysis of mtDNA from 120 yellowfin tuna with 12 restriction enzymes revealed a total of 34 genotypes, comprising 83 unique fragments. The most common genotype consisted of 52 fragments, representing a survey of 304 bp, or about 1.8% of the mtDNA genome. The mean size of the yellowfin tuna mtDNA genome, determined from the most common restriction fragment profiles for each of the 12 restriction enzymes, was 16,549 $\pm$ 309 bp (SD). Composite restriction morph fragment patterns and

fragment sizes are in Appendix I.

RFLP analysis of yellowfin tuna mtDNA demonstrated considerable variation. While four restriction enzymes, *Ava*I, *Dra*I, *Hin*dIII, and *Xho*I showed no variation, the remaining eight revealed two to seven restriction morphs each. Of the 34 genotypes, two were represented by 20 or more individuals, five were represented by four or more individuals, and 20 genotypes occurred only once (Table 1). Within-sample nucleon diversities were high and showed little variation among samples, ranging from 0.81 to 0.86, with a value of 0.84 for the pooled samples (Table 2). Mean nucleotide sequence diversities ranged from 0.26% to 0.37%, and averaged 0.31% among all samples (Table 2).

Despite the high level of within-sample variation, there was little evidence for genetic differentiation among the six sampling sites. The two most common genotypes (1 and 2) were observed at all locations at similar frequencies (30-40%, 10-25%, respectively; Table 1). Of the 12 other genotypes that occurred in more than a single individual, 11 were found in two or more samples, and only one (genotype 14) occurred in a single sample, represented by two individuals.

Pairwise estimates of corrected nucleotide sequence divergences between samples (including the Atlantic) were low, ranging from -0.000060% to 0.018%, with a mean pairwise divergence of -0.0015%. Similarly, estimates of G_{st} were low, ranging from 0.011 to 0.025, and values of $N_f m$ were correspondingly high, ranging from 19.5 to 44.5 females per generation. No clear pattern of phylogeographic structure was revealed in cluster analyses of nucleotide sequence divergences among mtDNA genotypes or

Table 1. Distributions of mtDNA genotypes among samples of yellowfin tuna, *Thunnus albacares*. Letters represent fragment patterns produced by the following enzymes (left to right): *Eco*RI, *Hin*dIII, *Pst*I, *Dra*I, *Ava*I, *Pvu*II, *Nci*I, *Ban*I, *Bcl*I, *Bgl*I, *Xho*I, and *Apa*I.

ID	Genotype	Sampling Location						Sum
		ECU	MEX	HAW	AUS	PNG	ATL	
1	AAAAAAAAAAAA	7	6	7	8	8	7	43
2	BAAAAAAAAAAA	2	5	3	4	3	3	20
3	BAAAAABAAAAA	3	-	1	-	1	1	6
4	AAAAAAAAECAA	1	1	-	2	-	2	6
5	AAAAAAAABAAA	-	-	-	1	1	2	4
6	AAAAAABAEAAA	1	-	1	-	-	1	3
7	AAAAAABABAAA	2	1	-	-	-	-	3
8	AABAAAAAAAAA	-	2	-	1	-	-	3
9	AAAAAAABAAAA	-	-	1	-	1	-	2
10	AAAAAAAABBAA	-	-	1	1	-	-	2
11	BAAAAAAAAAAB	-	-	1	-	-	1	2
12	BAAAAAAACAAA	-	-	1	-	1	-	2
13	AAAAAAAAEAAF	-	1	-	-	1	-	2
14	AAAAAABAAAAA	-	-	2	-	-	-	2
15	AAAAAAAAAAAB	-	-	-	1	-	-	1
16	AAAAABAAAAAA	-	-	-	-	-	1	1
17	AAAAAAAAAAAC	-	-	-	-	1	-	1
18	AAAAAAAAAAAD	-	-	-	-	1	-	1
19	AAAAAAAAFAAA	-	-	-	-	1	-	1
20	AAAAAAAAAAAF	-	-	1	-	-	-	1
21	AABAAAAABAAA	-	-	-	-	-	1	1
22	BABAAAAAAAAA	-	-	1	-	-	-	1
23	AAAAAABAAAAE	-	-	-	1	-	-	1
24	AAAAAAAADAAB	-	-	-	-	1	-	1
25	CAAAAABAAAAA	1	-	-	-	-	-	1
26	AABAAAAAAAAG	1	-	-	-	-	-	1
27	AABAAAAAEAAA	1	-	-	-	-	-	1
28	AAAAAABAAAAB	-	1	-	-	-	-	1
29	BAAAAAAAAAAD	-	1	-	-	-	-	1
30	BAAAAAAABBAA	-	1	-	-	-	-	1
31	BAAAAABABAAA	-	1	-	-	-	-	1
32	BAAAAABAGAAA	1	-	-	-	-	-	1
33	AAAAAABAADAA	-	-	-	-	-	1	1
34	AAAAAAACFEAA	-	-	-	1	-	-	1
Sum		20	20	20	20	20	20	120

Table 2. Genetic variation within samples of yellowfin tuna, *Thunnus albacares*, expressed as nucleon diversity (h) and percent nucleotide sequence diversity (π).

Sample (N)	Nucleon Diversity (h)	Nucleotide Sequence Diversity (π)
ECU (20)	0.863	0.373%
MEX (20)	0.863	0.345%
HAW (20)	0.868	0.290%
AUS (20)	0.816	0.304%
PNG (20)	0.837	0.262%
ATL (20)	0.863	0.321%
POOLED (120)	0.840	0.314%

sampling locations. Chi-square analysis of heterogeneity among samples was not significant, as randomizations of the data among the six samples were more heterogeneous than the observed genotypic distributions 581 of 1000 times ($p=0.581$).

To determine an appropriate number of individuals to examine from each location and number of restriction enzymes to employ, I compared levels of genetic variation and differentiation revealed by a pilot study of 12 individuals per location with 5 enzymes with larger analyses of up to 20 individuals per location with 12 enzymes (Table 3). Levels of variation were influenced more by increasing the number of enzymes surveyed than the number of individuals. Increasing the number of enzymes increased the number of genotypes, the nucleon diversities, and, to a lesser extent, nucleotide sequence diversities. Analysis of a greater number of individuals per location had little effect on diversity estimates with either 5 or 12 enzymes, although the ranges of within-sample diversities among locations decreased as more individuals were analyzed.

Increasing the number of individuals or the number of enzymes had little effect on levels of genetic differentiation. No significant differences were found in the distributions of genotypes among locations in Roff and Bentzen (1989) chi-square tests (Table 3). Furthermore, increasing the number of individuals in the 12 enzyme analysis did not increase the frequencies of genotypes unique to a location. Instead, many unique genotypes in the analysis of 12 individuals occurred in other locations as a greater number of individuals were used. Because increasing sample sizes from 12 to 20 individuals did not reveal greater spatial partitioning of genetic variation, it was concluded that 20 individuals was an appropriate sample size.

Table 3. Pooled percent nucleotide sequence diversities (π) and nucleon diversities (h), and respective within-sample ranges (in parentheses) obtained by varying the numbers of restriction enzymes and individuals of yellowfin tuna, *Thunnus albacares*, per sampling location. Probabilities of significance result from Roff and Bentzen (1989) chi-square analysis with 1000 randomizations of the data. The five enzymes selected for the pilot study are *Eco*RI, *Hin*dIII, *Pst*I, *Dra*I, and *Ava*I.

Design	Number of Genotypes	Nucleon Diversity (h)	Nucleotide Sequence Diversity (π)	Prob.
5 enzymes 12 individuals	5	0.496 (0.41-0.64)	0.248% (0.18-0.37%)	0.960
5 enzymes 20 individuals	5	0.509 (0.42-0.63)	0.258% (0.20-0.35%)	0.845
12 enzymes 12 individuals	27	0.829 (0.76-0.94)	0.318% (0.25-0.44%)	0.975
12 enzymes 20 individuals	34	0.840 (0.82-0.86)	0.314% (0.26-0.37%)	0.581

Discussion

Population structure is typically manifested as the spatial or temporal partitioning of genetic variation. Therefore, to demonstrate if population structure exists, a technique must first reveal a reasonable level of genetic variation. RFLP analysis of mtDNA revealed considerable genetic variation in yellowfin tuna. The overall nucleon diversity and mean nucleotide sequence diversity 0.84 is in the upper ranges reported for marine fishes (Avise et al., 1989; Ovenden, 1990; Gold and Richardson, 1991) including other large, pelagic fishes (Table 4), while the mean nucleotide sequence diversity (0.31%) was not high.

Genetic variability in tuna species has also been demonstrated by sequence analysis of a 307 base pair region of the mitochondrial cytochrome *b* gene of bluefin tuna, *Thunnus thynnus*; bigeye tuna, *T. obesus*; albacore, *T. alalunga*; and yellowfin tuna (Bartlett and Davidson, 1991). The sequence data they presented for 33 yellowfin tuna result in a nucleon diversity of h=0.28, a value that is considerably lower than the nucleon diversity of 0.84 obtained in the present study. Although nucleon diversity values are greatly influenced by the number of base pairs surveyed (Nei, 1987), the number examined in this study and in Bartlett and Davidson (1991) were very close (304 and 307, respectively). This difference in nucleotide sequence diversity may reflect a slower evolutionary rate for the mitochondrial cytochrome *b* gene relative to the entire mtDNA genome.

Significant genetic differentiation among yellowfin tuna from geographically distant locations was not found. Corrected nucleotide sequence divergences averaged only -0.0015%, indicating that the mean difference between two genotypes randomly chosen from any two samples was essentially the same as the difference between two genotypes randomly drawn

Table 4. Genetic variation within selected pelagic species determined by RFLP analysis of mtDNA employing 11-13 informative enzymes. Variation is expressed as nucleon diversity (h) and percent nucleotide sequence diversity (π).

Species	Nucleon Diversity (h)	Nucleotide Sequence Diversity (π)
Thunnus albacares[1] (Yellowfin Tuna)	0.84	0.91%
Thunnus alalunga[2] (Albacore Tuna)	0.60	0.31%
Tetrapturus audax[3] (Striped Marlin)	0.74	0.54%
Tetrapturus albidus[3] (White Marlin)	0.70	0.35%
Makaira nigricans[3] (Blue Marlin)	0.86	1.99%
Istiophorus platypterus[3] (Sailfish)	0.62	0.87%

[1]This study.
[2]Graves and Dizon (1989) and Graves, unpublished data.
[3]Graves and McDowell, 1993.

from the same sample. The frequencies of the two mostcommon genotypes were similar among all locations, and an overall chi-square test for heterogeneity was nonsignificant. Furthermore, nucleon diversities and nucleotide sequence diversities were similar among locations, although uniformity in the latter estimates was less pronounced.

The apparent genetic homogeneity of yellowfin tuna is consistent with the hypothesis that there is genetic exchange among locations. Rare mtDNA genotypes had low frequencies of occurrence (Table 1), characteristic of high gene flow (Slatkin, 1985), a situation similar to that found in other species for which high gene flow is suggested: American eel, *Anguilla rostrata* (Avise et al., 1986); marine catfishes, Ariidae (Avise et al., 1987); weakfish, *Cynoscion regalis* (Graves et al., 1992a); and bluefish, *Pomatomus saltatrix* (Graves et al., 1992b). Low pairwise estimates of G_{st} (0.011-0.025) also indicated homogeneity in yellowfin tuna, and gave rise to high N_em values (>19) which are consistent with high rates of gene flow among regions (Birky et al., 1983).

The absence of genetic differentiation between Pacific and Atlantic samples of yellowfin tuna is similar to that reported for other vagile pelagic species. RFLP analysis of mtDNA of skipjack tuna, *Katsuwonus pelamis*, and albacore from the Atlantic and Pacific oceans revealed modest amounts of within-sample variation, but no significant differentiation was found between Atlantic and Pacific conspecifics (Graves et al., 1984; Graves and Dizon, 1989). Similarly, RFLP analysis of mtDNA of the pelagic dolphin, *Coryphaena hippurus*, revealed no significant differentiation between samples from the Atlantic and Pacific oceans (Carol Reeb, University of Hawaii, personal communication). However, spatial partitioning of genetic variation occurs in at least some pelagic fishes.

Significant genetic differentiation was shown between Atlantic and Pacific blue marlin, *Makaira nigricans*, by direct sequence analysis of the mitochondrial cytochrome *b* gene (Finnerty and Block, 1992) and RFLP analysis of mtDNA (Graves and McDowell, 1993). Similarly, differentiation was found among striped marlin samples, *Tetrapturus audax*, and sailfish, *Istiophorus platypterus* from the Pacific Ocean by RFLP analysis of mtDNA (Graves and McDowell, 1993).

There are several characteristics of yellowfin tuna which could promote gene flow among locations. Yellowfin tuna are distributed circumtropically (Collette and Nauen, 1983) and occur around the Cape of Good Hope in the southern summer (Talbot and Penrith, 1962). Tagging studies demonstrated that adults are capable of traveling large distances between Pacific regions (Fink and Bayliff, 1970; Bayliff, 1984; Itano and Williams, 1992), and are capable of undergoing trans-Atlantic crossings (Bard and Scott, 1991). The existence of suitable spawning areas throughout the tropical oceans, suggested by circumtropical occurrence of larvae (Nishikawa et al., 1985), would permit unobstructed gene flow throughout the species' distribution.

Both genetic and morphological analyses revealed considerable variation in yellowfin tuna, however genetic analyses indicated that differentiation does not occur. Evidence that morphological characters are environmentally influenced was provided by comparison of the present data to the morphological differentiation found in Schaefer (1991, 1992). In the present analysis, 20 fish from four locations (Ecuador, Mexico, Hawaii, and Australia) were the same as those used in Schaefer's studies. Although no genetic differences were found among these locations, morphometric characters and gill-

raker counts differed significantly, which I conclude is the result of phenotypic plasticity. The finding of greater morphological variability among Pacific samples than occurred between the Atlantic and Pacific (Schaefer and Walford, 1950) also supports that phenotypic plasticity is the cause of morphological differentiation in the Pacific.

The null hypothesis that yellowfin tuna in the Pacific Ocean share a common gene pool could not be rejected in this analysis. These results were consistent with the alternate hypothesis that yellowfin tuna maintain sufficient gene flow among areas to prevent the accumulation of significant genetic differentiation. As theoretical models indicate that very low levels of migration (only a few individuals per generation) are needed to prevent genetic differentiation among large populations (Allendorf and Phelps, 1981; Hartl and Clark, 1989), the present data indicate only that some minimal amount of exchange is occurring. Other approaches are necessary to quantify the amounts of mixing among regions and to better judge whether or not separate stocks should be distinguished for management purposes.

Global Phylogeography of Mackerels of the genus *Scomber*

Introduction

Over the past decade many genetic analyses have provided an understanding of the population-genetic structure of several marine fishes (see Ovenden, 1990; Gold and Richardson, 1991; Avise, 1992), but few studies have been conducted of species that are cosmopolitan and have disjunct distributions. Iles and Sinclair (1982) proposed that physical oceanographic and geographic characteristics may have great influence on population structure, and that lower levels of genetic divergence among areas is likely to occur in species with long-lived, pelagic early life history stages. In addition, it has been suggested that little population-genetic structure is likely to occur in species that are highly mobile, allowing movement over large distances over the course of adult life (Rosenblatt, 1963; Hansen, 1978). These generalizations have resulted from observations of species distributions and have not been thoroughly evaluated by genetic analyses.

The few studies that describe genetic variation among geographically isolated populations of marine fishes have demonstrated that homogeneity can occur among populations separated by great distances. The minimal allozymic heterogeneity among samples of milkfish (*Chanos chanos*) separated by up to 10,000 km was attributed to wide-spread dispersal of pelagic larvae (Winans, 1980). Rosenblatt and Waples (1986) compared allozymic variation in 12 marine shore fishes sampled from both sides of the Pacific Barrier, an expanse of deep ocean separating central and eastern Pacific shallow water habitats (Ekman, 1953; Briggs, 1961). They concluded that the Pacific Barrier is

not a complete barrier to gene flow, as measures of genetic differentiation across the Pacific were an order of magnitude lower than estimates of conspecific populations of shorefishes from the Atlantic and Pacific sides of Panamá. Similarly, allozyme analysis of samples of the demersal spawning damselfish (*Stegastes fasciolatus*) demonstrated no significant differentiation throughout the 2,500 km Hawaiian archipelago (Shaklee, 1984). In addition, little allozymic differentiation was demonstrated within five demersally spawning damselfish species (*Chromis cyanea*, *Stegastes partitus*, *S. planifrons*, *S. leucostictus*, and *S. dorsopunicans*) sampled from isolated Caribbean reefs (Lacson, 1992).

In an effort to describe population-genetic patterns that occur in related species with varying distributional patterns, this study focuses on mackerels of the genus *Scomber*. These mackerels occur in widely-distributed populations of varying sizes on both sides of the tropics. Each of the three species of *Scomber* has a unique distribution, although sympatry among the species does occur (Figs. 2-4; Collette and Nauen, 1983). The chub mackerel, *S. japonicus*, is cosmopolitan, and distributed in coastal regions and adjacent seas of the Atlantic, Pacific, and northwest Indian oceans. The spotted chub mackerel, *S. australasicus*, occurs only in the Pacific, and the Atlantic mackerel, *S. scombrus*, only in the North Atlantic.

Similarities and differences occur among the three species of *Scomber* in spawning behavior and larval duration. *S. 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), while *S. japonicus* seems to spawn on average only 9 times in a season, and does

so only at night (Watanabe, 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 reach metamorphosis (about 15mm), which occurs in an additional 18-23 d and 16-23 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 stage as well. Little spawning or early life history data are available for *S. australasicus*.

Considerable information exists regarding the population structure of *S. scombrus*, and indicates that exchange between populations is sufficient to maintain genetic homogeneity. Two spawning groups in the northwest Atlantic, the "Northern contingent" in the southern Gulf of St. Lawrence, and the "Southern contingent" between Cape Cod and Cape Hatteras, were identified by Sette (1950) based on size composition and tagging data; however, these groups could not be discriminated by meristic, growth, or allozymic characters (MacKay and Garside, 1969; Maguire et al., 1987; Simard et al., 1992). 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 allozyme analysis (Jamieson and Smith, 1987).

Less is known of the population structure of *S. japonicus*. Matsui (1967) showed that phenotypic variation was greater 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 non-overlapping distributions of gill raker counts, although other variable morphological characters of Atlantic fish were similar; members

of all populations had 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). Electrophoretic analysis of 4 polymorphic loci revealed no evidence for differentiation among samples of *S. japonicus* from the southeast Atlantic off Namibia (Zenkin and Lobov, 1989), but heterogeneity in immunological reactivity indicated evidence of population structure off the northwest African coast (Weiss, 1980). Differences in growth rates and morphology 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 elucidate the inter- and intraspecific genetic relationships within mackerels of the genus *Scomber*, this study evaluated restriction fragment length polymorphisms (RFLPs) of mitochondrial DNA (mtDNA). This method of genetic analysis has proven useful to reveal phylogeographic structure within a variety of marine and freshwater fish species (Avise, 1992). Because the mtDNA molecule is clonally inherited, information regarding historical phylogenetic relationships are retained, hence analysis of mtDNA can render considerable information of historical relationships among populations and the mtDNA lineages they possess.

Materials and Methods

Specimen Collection

Collections of up to 21 specimens each of *S. scombrus*, *S. australasicus*, and *S. japonicus* were obtained with the assistance of regional scientists. Whole fish were

frozen and shipped to the laboratory on dry ice. Sample names, locations, and sizes are presented in Table 5. Specimens of *Rastrelliger kanagurta*, the sister group to *Scomber* (Collette et al., 1984), were obtained from Sri Lanka.

MtDNA Preparation and Analysis

MtDNA enriched genomic DNA was isolated from 3 g of lateral red muscle or whole young-of-year with digestive tracts removed (A-NZL only) following Chapman and Powers (1984), modified by the omission of sucrose step gradients and the use of 1.5% sodium dodecyl sulfate for mitochondrial lysis. Isolated 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 Bethesda Research Laboratories) 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 biotin-labeled probe as described previously (Scoles and Graves, 1993 [previous chapter]). Probe consisted of the entire yellowfin tuna, *Thunnus albacares*, mtDNA molecule in four cloned fragments ligated in pBluescript SK- at the *Pst*I site. MtDNA fragments were visualized by using the BRL BluGene Non-Radioactive Nucleic Acid Detection Kit.

A 12-letter composite mtDNA haplotype (genotype), indicating the fragment pattern for each enzyme, was developed for each individual. Letters were assigned to restriction morphs as they were encountered, beginning with 'A' for the closely related *S. japonicus* and *S. australasicus* group, and proceeding through the alphabet, and beginning with 'Z'

Table 5. Sample sizes and locations and names of *Scomber japonicus*, *S. australasicus*, and *S. scombrus*. Sample names include species collected (first letter), followed by a three letter code for location.

Sample name	Size	Location
Scomber japonicus		
J-FLA	20	Panama City, Florida
J-ARG	18	Mar del Plata, Argentina
J-ISR	20	Mediterranean coast of Israel
J-IVC	20	Abidjan, Ivory Coast
J-SAF	20	Cape Town, South Africa
J-RED	15	South of Sinai Peninsula, Red Sea
J-TWN	20	Kaohsing, Taiwan
J-JPN	20	Tokyo, Japan
J-CAL	20	San Diego, California
Scomber australasicus		
A-AUS	18	New South Wales, Australia
A-NZL	19	Wellington, New Zealand
A-JPN	21	Tokyo, Japan
A-MEX	20	Revillagigedo Islands, Mexico
Scomber scombrus		
S-MAS	20	Boston, Massachusetts
S-ENG	20	Plymouth, England
Total	291	All Areas

and proceeding in reverse alphabetical order for *S. scombrus*. For the few *S. scombrus* restriction morphs that also occurred in *S. japonicus* or *S. australasicus*, the latter species' restriction morph letter designation was used.

Estimates of nucleon diversity (*h*) were calculated by following Nei (1987), and of nucleotide sequence diversity (π) by following Nei and Tajima (1981), using the mtDNA statistical package REAP (McElroy et al., 1992). Because of the considerable level of divergence of *S. scombrus* from *S. japonicus* and *S. australasicus*, separate presence/absence matrices of restriction sites, generated from restriction fragment patterns, were used in statistical comparisons among populations. A single presence/absence matrix of restriction fragments was used in a comparison between *S. scombrus* and the pooled data of *S. japonicus* and *S. australasicus*. Estimates of nucleotide sequence divergence (*d*) among haplotypes were determined by following 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 corrected nucleotide sequence divergences (δ) among populations were calculated by following Nei and Li (1979), using REAP.

Corrected nucleotide sequence divergences were clustered by the unweighted pair-group method with arithmetic means (UPGMA) by using the average linkage algorithm of the SPSS-X statistical package (Norusis, 1988). Using a single presence/absence matrix of restriction sites, parsimony analysis among genotypes, rooted to *S. australasicus*, was conducted using the software package Hennig86 (Farris, 1988).

Significance levels of tree branches of the resulting strict consensus of equally parsimonous trees were obtained by bootstrapping using the BOOT option of Random Cladistics (Siddall, 1994) with 1000 replications. Chi-square analyses among samples were conducted by using the Monte-Carlo method of Roff and Bentzen (1989) with 1000 randomizations of the data using REAP.

DNA Amplification and Sequence Analysis

Upon identification of the major mtDNA matrilines, one individual from each was selected for DNA sequence analysis, in addition to one individual of *Rastrelliger kanagurta*. A 418 bp region of the cytochrome *b* gene was amplified by the polymerase chain reaction (PCR) using 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 using the Amplitaq Kit (Perkin-Elmer/Cetus) in 100 ul 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, 1 mg template DNA, and 2.5 units taq polymerase. In a Perkin-Elmer/Cetus thermocycler, 30 cycles were conducted of 1 min denaturation at 94°C, 1 min annealing at 50°C, and 3 min extension at 72°C. PCR product from each individual was ligated to vector pNoTA by using the Prime PCR Cloner kit (5 Prime → 3 Prime, Inc.), and plasmid was prepared from *E. coli* host strain JM109 by alkali lysis (Sambrook et al., 1989). Sequencing was accomplished using the Sequenase kit (United States Biochemical), using New England Biolab's universal primers nos. 1211 and 1201,

and both the heavy and light strands were read. A cladistic tree among the cytochrome *b* sequences, rooted to *Rastrelliger kanagurta*, was developed using the ALLTREES option of the software package PAUP (Swofford, 1985).

Results

MtDNA restriction site analysis of 40 *Scomber scombrus* revealed 13 unique genotypes and a total of 56 restriction sites, of which 16 were polymorphic. Among the genotypes of *S. scombrus* nucleotide sequence divergences ranged from 0.17% to 0.86%. On average 294 bp were 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 $\pm$ 213 bp (SD). Restriction site analysis of 246 *S. japonicus* and *S. australasicus* revealed a total of 93 unique genotypes, including 86 restriction sites, of which 59 were polymorphic. Among genotypes of *S. japonicus* and *S. australasicus*, nucleotide sequence divergences ranged from 0.15% to 2.9%. On average, 310 bp were surveyed per individual, representing 1.85% of the mtDNA genome. The average size estimate of the mtDNA genome of *S. japonicus* or *S. australasicus*, 16,781 $\pm$ 195 bp (SD), was not statistically different than that of *S. scombrus*.

Genetic variability was lower in *S. scombrus* relative to the other three species (Table 6). S-ENG was the least variable sample in the study (h=0.28), with a total of 4 genotypes of which 3 occurred only once in the sample. In S-BMA 10 genotypes were revealed, with three at elevated frequencies (Table 7). The overall nucleon diversity for

Table 6. Genetic variation within samples of *Scomber japonicus*, *S. australasicus*, and *S. scombrus*, expressed as nucleon diversity (± standard error) and percent nucleotide sequence diversity.

Sample (N)	Nucleon diversity (h)	Percent nucleotide sequence diversity (π)
Scomber japonicus		
J-FLA (20)	0.933 ± 0.054	0.400
J-ARG (18)	0.895 ± 0.044	0.502
J-ISR (20)	0.895 ± 0.052	0.383
J-IVC (20)	0.910 ± 0.042	0.393
J-SAF (20)	0.810 ± 0.092	0.383
J-RED (15)	0.952 ± 0.034	0.410
J-TWN (20)	0.858 ± 0.045	0.291
J-JPN (20)	0.879 ± 0.043	0.349
J-CAL (21)	0.638 ± 0.098	0.135
Scomber australasicus		
A-AUS (18)	0.863 ± 0.064	0.754
A-NZL (19)	0.748 ± 0.075	0.769
A-JPN (21)	0.810 ± 0.080	0.300
A-MEX (15)	0.595 ± 0.098	0.131
Scomber scombrus		
S-MAS (20)	0.853 ± 0.063	0.288
S-ENG (20)	0.284 ± 0.128	0.069

Table 7. Distributions of mtDNA genotypes 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.

ID	Genotype	J-TWN	J-JPN	Sum
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

ID	Genotype	J-CAL	Sum
14	BAIABIADEAAA	12	12
15	BAIABHADEAAA	5	5
16	BAIABIADEAAB	1	1
17	BAIABIDDEAAA	1	1
18	BAIADIADEAAA	1	1
19	BAJABIADCAAA	1	1

Table 7, cont.

ID	Genotype	J-FLA	J-ARG	J-SAF	J-IVC	J-ISR	Sum
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	AAAAAAABAABAA			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
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

ID	Genotype	J-RED	Sum
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

Table 7, cont.

ID	Genotype	A-NZL	A-AUS			Sum
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

ID	Genotype	A-NZL	A-AUS	A-MEX	A-JPN	Sum
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
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

ID	Genotype	S-ENG	S-BMA	Sum
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

S. scombrus, 0.58, was lower than that of any of the samples of *S. japonicus* (h=0.64-0.95) or *S. australasicus* (h=0.59-0.86). Three restriction enzymes, *Pvu*II, *Bgl*I, and *Bsu*36I, were invariant in *S. scombrus*, while the remaining 9 restriction enzymes revealed from 2 to 5 fragment patterns (Appendix II).

A wide range of diversities was observed among samples of *S. australasicus* and *S. japonicus*. 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 most other samples, possessing only 4 and 6 genotypes, respectively. The highest genotypic diversity occurred J-RED (h=0.95), with 5 of 10 genotypes represented twice; and the highest nucleotide sequence diversities occurred in A-NZL (π=0.77%) and A-AUS (π=0.75%) due to the presence of two divergent mtDNA matrilines within each sample. With the exception of *Bgl*I in *S. australasicus*, all restriction enzymes were variable in both species, revealing 2 to 7 fragment patterns in *S. australasicus*, and 2 to 15 in *S. japonicus*. The enzyme *Apa*I revealed the greatest number of variants in both species (Appendix II).

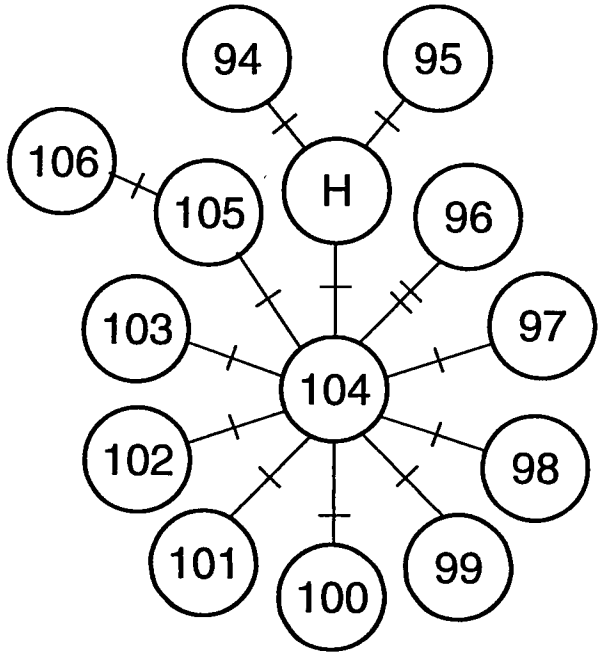
Scomber scombrus was highly divergent from *S. australasicus* and *S. japonicus*. Comparison between *S. scombrus* and the pooled data of *S. japonicus* and *S. australasicus* resulted in an estimate of net nucleotide sequence divergence of 11.9% (estimated from restriction fragment, not site data). The corrected mean nucleotide sequence divergence between *S. japonicus* and *S. australasicus*, 1.21%, indicated these species are much more closely related to one another than to *S. scombrus*. Of the 105 restriction morphs represented by the 3 species for the 12 enzymes, 2 were shared

between *S. scombrus* and *S. australasicus*, 1 was shared between *S. scombrus* and *S. japonicus*, and 22 were shared between *S. australasicus* and *S. japonicus*.

Considerable intraspecific differentiation was revealed in *S. scombrus*. Only one genotype (#94) was shared between the samples, occurring at very different frequencies in each (0.35 in S-ENG, and 0.85 in S-MAS; Table 7). A test of homogeneity between samples was highly significant ($P<0.001$), although the estimate of net nucleotide sequence divergence between S-ENG and S-MAS was low ($\delta=0.011\%$), reflecting the close relationship among genotypes (Fig. 7).

Varying levels of differentiation among samples were observed in *S. australasicus*. Samples from Australia and New Zealand were very similar. Three genotypes, #69, #79, and #80, occurred with similar frequencies in both samples (Table 7), and no heterogeneity was revealed between the samples ($P=0.718$, $\delta=-0.019\%$). These samples were genetically distinct from A-JPN and A-MEX in which a total of twelve genotypes were revealed, of which two were shared, and only one (#79) occurred in a large number of individuals in both samples (9 and 12, respectively). Three other genotypes (#82-84) were unique to either A-JPN or A-MEX, but not rare. Although the net nucleotide sequence divergence between A-JPN and A-MEX was small ($\delta=0.02\%$), a test for homogeneity suggested these groups are independent genetic units ($P=0.013$). The mean corrected nucleotide sequence divergence between A-JPN and A-MEX pooled, and A-AUS and A-NZL pooled, was high ($\delta=0.54\%$). Only one genotype was shared between these two groups (#79) which occurred in different frequencies (0.51 and 0.05, respectively), and a test of heterogeneity was highly significant ($P<0.001$).

Figure 7. Wagner network illustrating the number of restriction site differences and relationship among mtDNA genotypes of *Scomber scombrus*. Slashes represent the number of restriction site differences between mtDNA genotypes. One hypothetical genotype is indicated by the letter 'H.'


94
95
106
H
105
96
103
97
104
102
98
101
100
99

Divergence was demonstrated between populations of *S. japonicus* from the eastern and western regions of the Pacific. Samples from Japan (J-JPN) and Taiwan (J-TWN) shared 4 genotypes, 3 of which were in a number of individuals (#s 1-3, Table 7). No significant heterogeneity was observed between these samples (P=0.658, δ=-0.010%). However, a comparison of the pooled data of J-JPN and J-TWN with *S. japonicus* collected from across the eastern Pacific (J-CAL) revealed 1 fixed restriction site difference between the regions, and a net nucleotide sequence divergence of 0.30%.

In *S. japonicus* from the Atlantic and Mediterranean Sea, many closely related genotypes were observed, several of which were shared among many samples (Table 7). No heterogeneity was observed among J-ISR, J-IVC, and J-SAF, from the eastern Atlantic (P=0.451, δ=-0.003% to 0.015%, Table 8, Table 9), or between J-IVC and J-ARG across the Atlantic (P=0.07, δ=0.042%). All other tests of homogeneity among Atlantic samples were significant (Table 8).

The Red Sea sample was more closely related to the Atlantic group than the Pacific group, but divergent from both. The mean corrected nucleotide sequence divergence between J-RED and the pooled Atlantic samples was 0.73%, while 1.1% sequence divergence occurred between J-RED and the pooled Pacific samples. The mean corrected nucleotide sequence divergence between Atlantic and Pacific *S. japonicus* was 1.4%.

Phenetic and parsimony analyses indicated a close relationship among some groups of *S. japonicus* and *S. australasicus* genotypes. UPGMA cluster analysis of all 93 genotypes of *S. japonicus* and *S. australasicus* revealed 3 clusters corresponding to Atlantic, Pacific, and Red Sea *S. japonicus*. Two clusters were revealed in *S.*

Table 8. Probabilities of significance from Roff and Bentzen's (1989) chi-square analysis with 1000 randomizations of the data of samples of A) Atlantic *Scomber japonicus*, B) Pacific *S. japonicus*, C) *S. australasicus*, and D) *S. scombrus*. Comparisons subsequent to the first of each categorie are protected level tests, and the values of α were not adjusted.

Comparison	Chi-Square	Number of Simulations Exceeding Chi-Square	Prob.
A) 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-ISR J-SAF	20.60	294	0.343^{NS}
J-FLA J-ARG	28.37	1	0.001^{**}
J-ISR J-IVC J-SAF J-FLA	113.55	2	0.002^{**}
J-ISR J-IVC J-SAF J-ARG	108.11	15	0.015^{*}
J-ISR J-IVC J-ARG	54.76	43	0.043^{*}
J-ISR J-SAF J-ARG	64.77	4	0.004^{**}
J-IVC J-SAF J-ARG	60.84	28	0.028^{*}
J-ISR J-IVC J-FLA	58.40	16	0.016^{*}
J-ISR J-SAF J-FLA	74.90	0	$<0.001^{**}$
J-IVC J-SAF J-FLA	65.81	10	0.010^{*}
J-ARG J-ISR	23.03	32	0.033^{*}
J-ARG J-IVC	22.21	68	0.070^{NS}
J-ARG J-SAF	26.89	0	0.001^{**}
J-FLA J-ISR	32.00	1	0.001^{**}
J-FLA J-IVC	24.33	14	0.014^{*}

J-FLA J-SAF	35.33	0	<0.001**

Table 8, cont.

Comparison	Chi-Square	Number of Simulations Exceeding Chi-Square	Prob.
B) Pacific *Scomber japonicus*			
J-JPN J-TWN	10.67	658	0.658[NS]
C) *Scomber australasicus*			
A-JPN A-MEX A-AUS A-NZL	128.94	0	<0.001**
A-JPN A-MEX A-AUS	80.00	0	<0.001**
A-JPN A-MEX A-NZL	81.52	0	<0.001**
A-AUS A-NZL A-JPN	69.20	0	<0.001**
A-AUS A-NZL A-MEX	67.45	0	<0.001**
A-JPN A-MEX	17.75	13	0.013*
A-AUS A-NZL	11.67	718	0.718[NS]
A-JPN A-AUS	34.30	0	<0.001**
A-JPN A-NZL	35.38	0	<0.001**
A-MEX A-AUS	36.39	0	<0.001**
A-MEX A-NZL	35.31	0	<0.001**
D) *Scomber scombrus*			
S-ENG S-MAS	20.17	0	<0.001**

*Significantly heterogeneous at $\alpha=0.05$.
**Significantly heterogeneous at $\alpha=0.01$.
[NS]Not significant.

Table 9. Percent net nucleotide sequence divergences among samples of A) *Scomber scombrus*, B) *Scomber australasicus* and *Scomber japonicus*.

A) *Scomber scombrus*

	S-MAS	S-ENG
S-MAS	———	
S-ENG	0.011	———

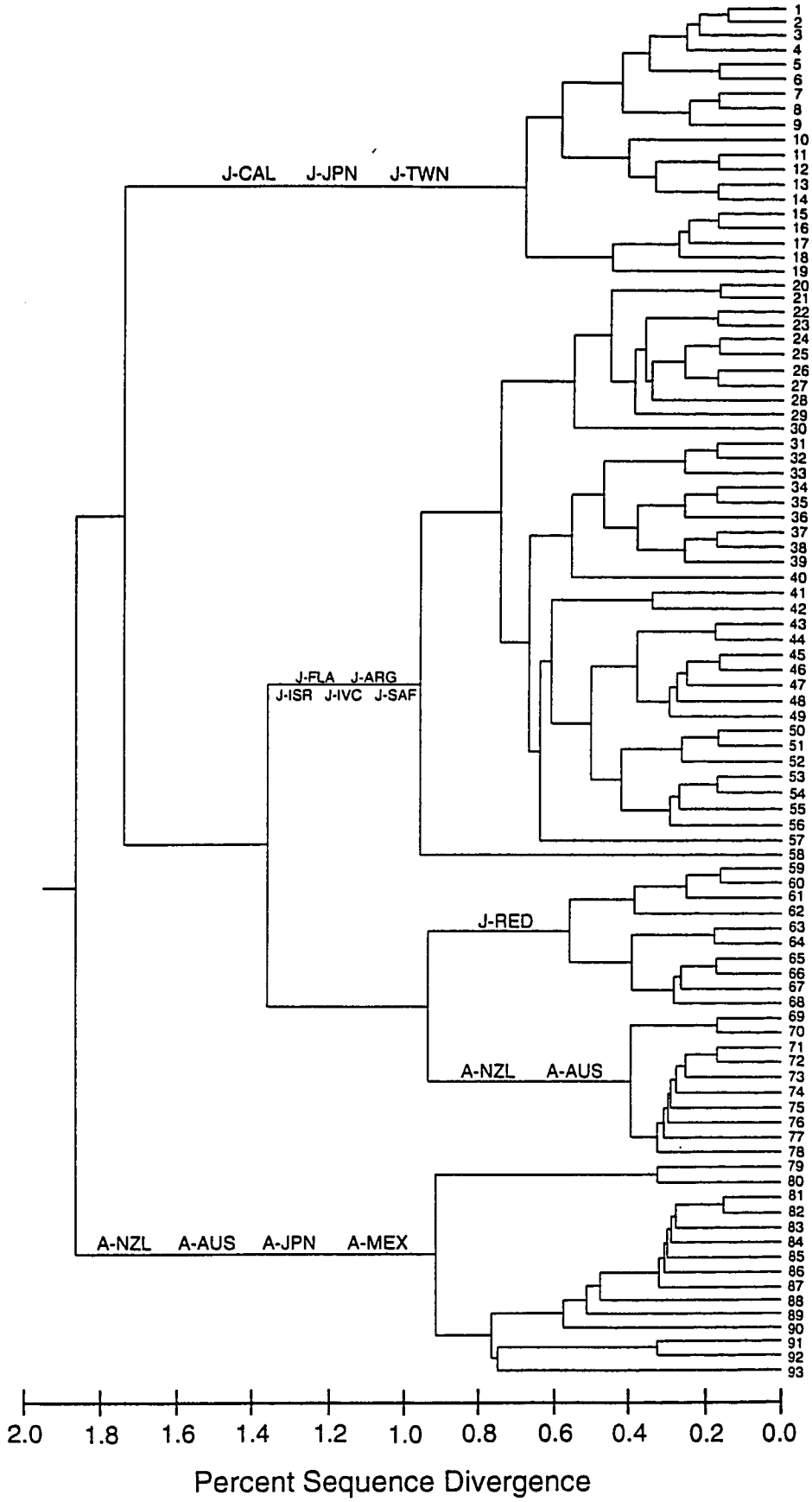
B) *Scomber australasicus* and *Scomber japonicus*

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

Atlantic, Pacific, and Red Sea *S. japonicus*. Two clusters were revealed in *S. australasicus*, one that was found only in samples from New Zealand and Australia, termed "unique," and one that included individuals of all 4 sample sites of this species, termed "ubiquitous" (Fig. 8). Phenetic cluster analyses of genotypes (Fig. 8) and samples (Fig. 9) suggested a close relationship between the unique New Zealand/Australia cluster and *S. japonicus* from the Red Sea. A strict consensus among 1031 equally parsimonious trees of genotypes of *S. japonicus* and *S. australasicus*, rooted to genotypes of *S. australasicus*, revealed that *S. japonicus* is monophyletic with the unique New Zealand/Australia lineage (Fig. 10), but the relationship among this lineage and the lineages of *S. japonicus* was not resolved. However, cladistic analysis of cytochrome *b* sequences indicated a paraphyletic relationship in *S. japonicus* (Fig. 11). The matrix of restriction site characters used in the parsimony analysis is in Appendix III, the cytochrome *b* sequences are in Appendix IV, and the 77 informative characters resulting from the removal of primer sequences, characters with missing values, and those that were uninformative are in Appendix V.

To determine if the unique and ubiquitous lineages of *S. australasicus* represented distinct, cryptic species, 6 individuals of each lineage were compared by allozyme analysis. Using red muscle and following Murphy et al. (1990) for horizontal starch gel electorphoresis, four enzyme systems (malate dehydrogenase $NADP^+$, *Mdhp*; lactate dehydrogenase, *Ldh*; isocitrate dehydrogenase, *Idh*; and glucose-6-phosphate dehydrogenase, *G6pdh*) were resolved in Tris-citrate buffer (electrode buffer: 0.687M Tris, 0.157M citric acid, pH 8.0; gel buffer: 1:29 dilution of electrode buffer) and one

Figure 8. Cluster of nucleotide sequence divergences among mtDNA genotypes of *Scomber japonicus* and *S. australasicus*.


J-CAL J-JPN J-TWN
J-FLA J-ARG
J-ISR J-IVC J-SAF
J-RED
A-NZL A-AUS
A-NZL A-AUS A-JPN A-MEX
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
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68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Percent Sequence Divergence

Figure 9. Cluster of nucleotide sequence divergences (Table 9) among samples of *Scomber japonicus* and *S. australasicus*.

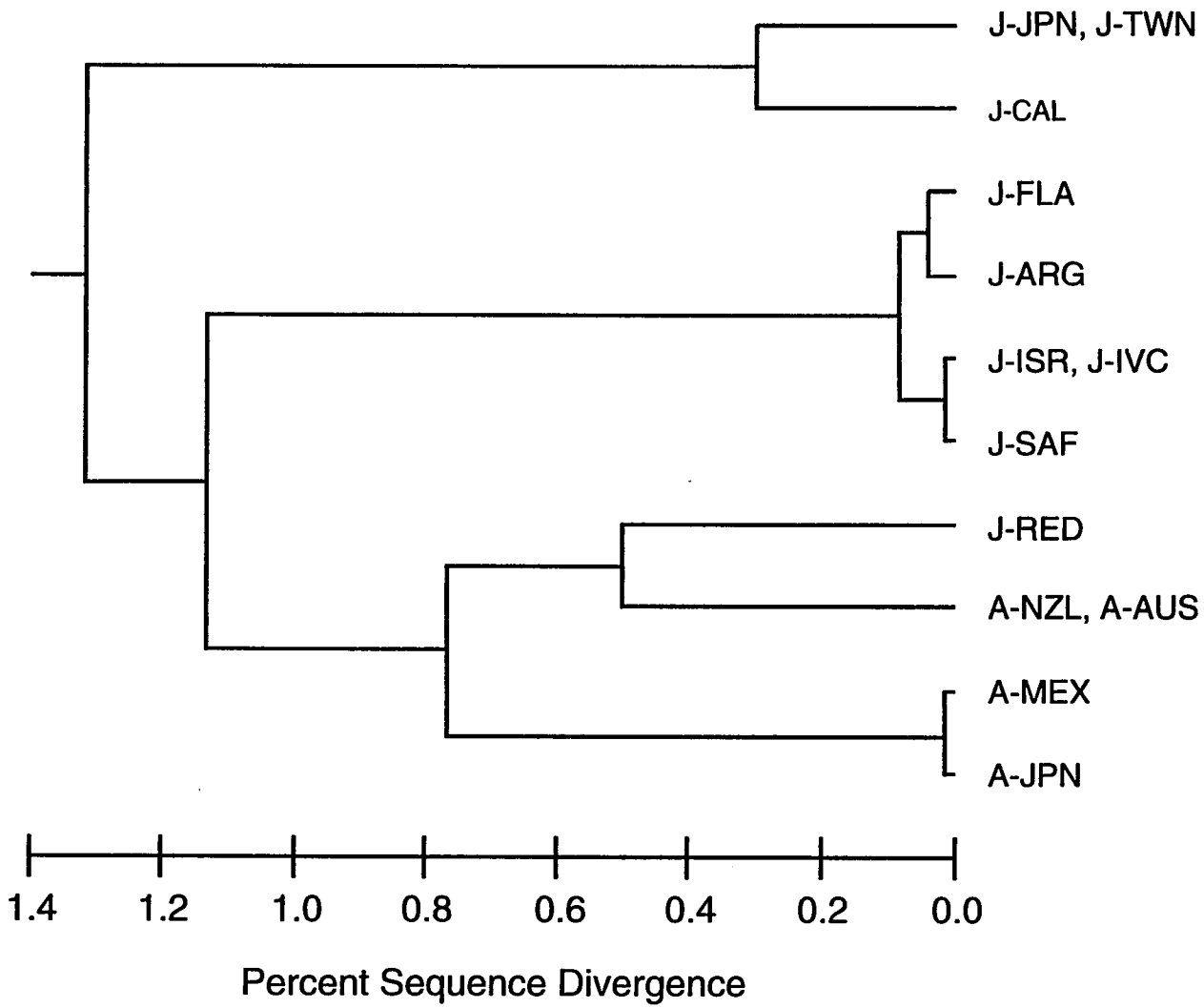

J-JPN, J-TWN
J-CAL
J-FLA
J-ARG
J-ISR, J-IVC
J-SAF
J-RED
A-NZL, A-AUS
A-MEX
A-JPN
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Percent Sequence Divergence

Figure 10. Strict consensus of 1031 equally parsimonous trees of genotypes of *Scomber japonicus* and *S. australasicus*, rooted to genotypes of *S. australasicus*. Significance of tree branchs were determined by bootstrapping with 1,000 replications. The tree length is 117 steps, and the consistency index is 0.49.

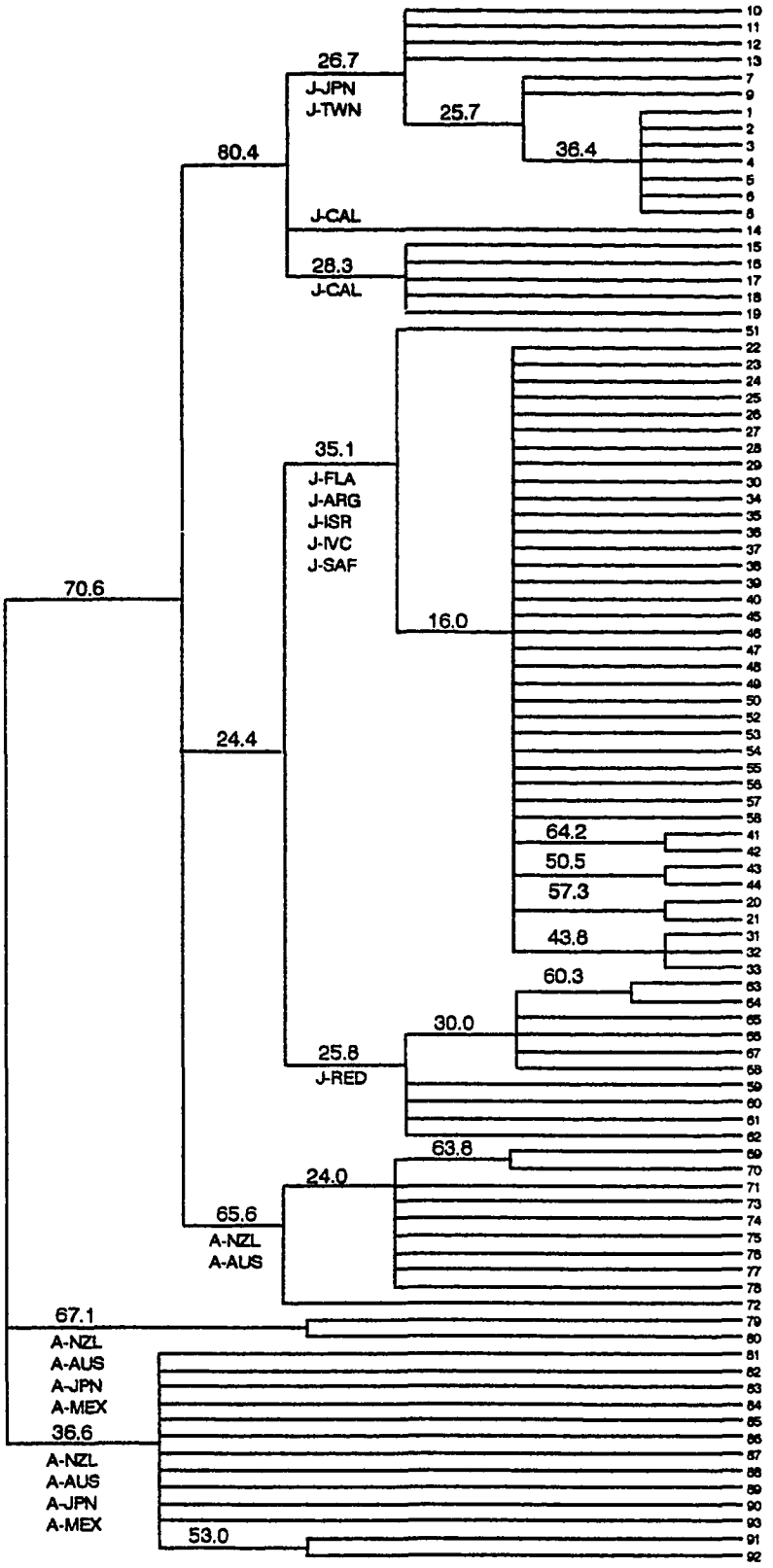
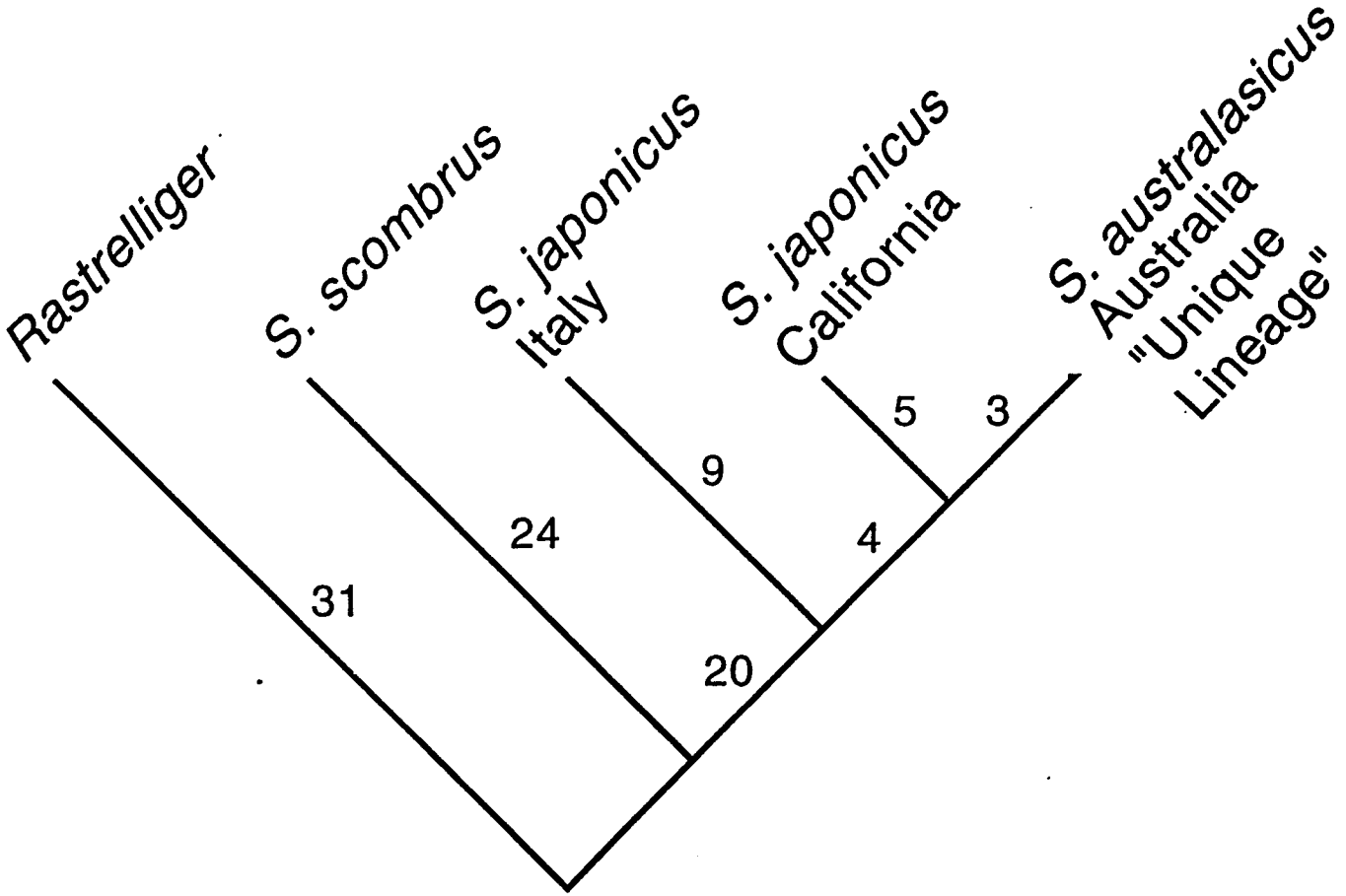

26.7
J-JPN
J-TWN
25.7
36.4
80.4
J-CAL
28.3
J-CAL
35.1
J-FLA
J-ARG
J-ISR
J-IVC
J-SAF
70.6
16.0
24.4
64.2
50.5
57.3
43.8
60.3
30.0
25.8
J-RED
63.8
24.0
65.6
A-NZL
A-AUS
67.1
A-NZL
A-AUS
A-JPN
A-MEX
36.6
A-NZL
A-AUS
A-JPN
A-MEX
53.0

Figure 11. Cladistic relationship among major lineages and *S. scombrus*, rooted to *Rastrelliger kanagurta*, with branch lengths indicated. There were 77 informative characters of the cytochrome *b* sequence used to draw the tree which is 96 steps in length.


Rastrelliger
S. scombrus
S. japonicus
Italy
S. japonicus
California
S. australasicus
Australia
"Unique
Lineage"
31
24
9
5
3
4
20

(malate dehydrogenase, *Mdh*) was resolved in TE (electrode buffer:0.1M Tris, 4.5mM EDTA, pH 9.6; gel buffer: 1:9 dilution of electrode buffer). In *Mdh*, two loci were observed and all individuals were fixed for a single allele except for 1 of the ubiquitous lineage that was heterozygous. All other enzymes revealed 2 loci and were fixed for a common allele in fish of both lineages with no heterozygosity observed.

Discussion

Generic level taxonomy

Early studies of *Scomber* species demonstrated considerable interspecific morphological divergence. 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 similar 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).

The level of genetic divergence of *S. scombrus* from *S. australasicus* and *S. japonicus* was high, and is not inconsistent with a generic level taxonomic status between these two groups. Other intergeneric estimates of nucleotide sequence divergence in salmonids between *Onchorynchus* and *Salmo* (9.0-14.2%), *Onchorynchus* and *Salvelinus* (11.4-12.4%), and *Salmo* and *Salvelinus* (13.4-18%) were similar to that observed in *Scomber* (Gyllensten and Wilson, 1986). However, estimates of interspecific divergence in *Paralabrax* were also on the order of that observed in *Scomber*. Pairwise comparisons

of *Paralabrax clathratus*, *P. nebulifer*, and *P. maculatofasciatus* revealed estimates of nucleotide sequence divergence ranging from 6.9% to 14.5% (Graves et al., 1990). Because mtDNA is inherited clonally, divergent lineages can persist even within a single population, as seen in *S. australasicus*. The observed value of nucleotide sequence divergence of *S. scombrus* from *S. japonicus* and *S. australasicus* is within the range known for other congeneric species, and does not refute the current taxonomy.

Species level taxonomy

The current taxonomy of *Scomber* is based on morphological characters. Species can be distinguished by the number of interneural bones under the first dorsal fin, length of the space between the dorsal fins relative to that of the dorsal groove, and relative positions of the anal and dorsal fin origins (Matsui, 1967). Genetic data supported the specific status of *S. australasicus* and *S. japonicus*. Allozyme electrophoresis revealed several fixed allelic differences between *S. australasicus* and Pacific *S. japonicus*, and provided no evidence for the hybrid origin of individuals of intermediate color morphology (Kijima et al., 1986).

The systematic status of *S. japonicus* was not as clear, as this species is characterized by considerable morphological variability. Several names have been used for the groups occurring in different areas: *S. diego* (Ayres, 1857), eastern North Pacific; *S. peruanus* (Jordan and Hubbs, 1925), eastern South Pacific; *S. japonicus* (Houttuyn, 1782), western Pacific; *S. colias* (Gmelin, 1789) Mediterranean to South Africa; *S. grex* (Mitchell, 1815), Massachusetts to Venezuela, and Argentina.

Differences in pigmentation, tooth crenulation, scale size, and gill raker counts on the lower 1st arch vary among fish from the Pacific, western Atlantic, and eastern Atlantic. These groups were synonymized into the single species *S. japonicus*, because, as stated by Matsui (1967), "[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."

Mitochondrial DNA analysis did not refute the specific status of *S. japonicus* and *S. australasicus*, as determined by Matsui (1967). Parsimony analysis revealed that the mtDNA matrilines of *S. japonicus* and the unique *S. australasicus* matriline are monophyletic. Although the relationships within this group was unresolved, this clade is clearly differentiated from the ubiquitous matriline of *S. australasicus*. In *S. australasicus* two highly divergent lineages that differed by an average of 1.84% nucleotide sequence divergence were present. The nucleotide sequence divergence between these lineages was greater than that between any other pairs of genotype clusters or samples of *S. japonicus* or *S. australasicus*. Although the estimate of nucleotide sequence divergence between the two *S. australasicus* lineages was high, the data do not suggest species-level differentiation. Allozyme analysis of individuals of each of the two lineages revealed 10 loci that were all but for one fish, fixed for a single allele providing no evidence of divergence.

Phylogeographic patterns--the population level

The distribution of genotypes between eastern and western Atlantic samples of *S.*

scombrus indicated the populations do not share a common gene pool. The close relationship among genotypes in these samples (Fig. 7) contrasts with the finding of two divergent mtDNA matrilines (δ=3.7%) between samples of capelin, *Mallotus villosus*, from eastern and western regions of the North Atlantic (Dodson et al., 1991). Isolation between populations of *S. scombrus* was probably more recent and may be a function of this species' greater vagility.

In Atlantic *S. japonicus*, genotype distributions were significantly different between western samples, but not among eastern samples. Unique genotypes occurred within each of the western Atlantic samples (J-FLA and J-ARG) at relatively high frequencies (10-20%), and a test of homogeneity was significant. The tropical western Atlantic appears to be a barrier to gene flow in *S. japonicus*. In contrast, no significant differentiation was observed among distributions of genotypes in samples from the eastern Mediterranean (J-ISR), Ivory Coast (J-IVC), and South Africa (J-SAF). There appears to be no barrier to gene flow in *S. japonicus* between northern and southern regions of the east Atlantic. The tropical water of the east Atlantic occupies the smallest area of all the tropical regions, and temperate species have been known to cross this region by moving under the warm water mass, thereby achieving an antitropical distribution (Briggs, 1974).

Homogeneity tests among samples across the Atlantic were significant, except for the pairwise comparison of J-ARG and J-IVC (Table 8). Genetic similarity between these samples may reflect recent isolation, or gene flow. The Gulf of Guinea region and the northernmost extension of the southwest Atlantic *S. japonicus* population are

separated by the most narrow region of the Atlantic, and it may be possible that larval exchange occurs between these regions assisted by trans-Atlantic equatorial currents, or that there is exchange of adults.

East and west Atlantic populations of *S. japonicus* are morphologically differentiated. Starks (1921) and Matsui (1967) showed that the distributions of gill raker counts on the lower first arch of east and west Atlantic samples did not overlap. While these differences may reflect genetic divergence, meristic characters are sometimes known to be phenotypically plastic (Taning, 1950; Lindsey, 1954, 1958). It was also previously suggested that groups of *S. japonicus* off Namibia, among which allozyme analysis revealed no significant genetic differences, differ in coloration, which can also be environmentally influenced (Zenkin and Lobov, 1989).

The sample of *S. japonicus* from the Red Sea (J-RED) possessed the highest genotypic diversity estimate in the study, and was more closely related to Atlantic than Pacific *S. japonicus*. The relationship to Atlantic samples may reflect historical contact between the populations of the Red Sea and South Africa during times when equatorial Indian Ocean temperatures were lower than those of the present day, allowing gene flow through this region.

This study provides no evidence of gene flow in *S. japonicus* through the Suez Canal, although several species are known to have made the passage, primarily from the Red Sea to the Mediterranean (Ben-Tuvia, 1978). *Rastrelliger kanagurta*, a close relative to *Scomber*, is among those known to have invaded the Mediterranean from the Red Sea (Collette, 1969). The samples of *S. japonicus* from the eastern Mediterranean (J-ISR) and

the Red Sea were differentiated by 0.72% nucleotide sequence divergence, and 1 fixed restriction site difference, and an additional site that was nearly fixed. However, this does not exclude the possibility of movement through the Suez Canal by *S. japonicus*, which may be revealed by greater sampling effort.

In *S. japonicus* from the Pacific Ocean, genotype frequencies in samples from the northwest region (J-JPN and J-TWN) were similar, but shared no genotypes with the sample from the western Pacific (J-CAL). Fishery data indicates *S. japonicus* in the region of Japan and Taiwan represent separate populations with unique spawning grounds and larval retention areas (Sato, 1990). However, the data of the present study are consistent with exchange among populations. Whether exchange is the result of larval drift or adult movement is indeterminable, however movement of adults of *S. australasicus* is known to occur between these regions (Chang and Wu, 1977).

Samples of *S. australasicus* between New Zealand and Australia, regions are separated by nearly 2,000 km of deep ocean, were genetically homogeneous. This result indicated that the potential for genetic exchange in *S. australasicus* is high, which was also suggested by comparison of *S. australasicus* samples from the northern Pacific. While 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 east and west Pacific samples of *S. japonicus*.

The lowest genotypic and nucleotide sequence diversity in the study occurred in A-MEX, collected from the Revillagigedo Islands, and may indicate the origination of this

population via a founder event from a source population in the northwest Pacific region, or Hawaii. Colonization of the Revillagigedo Islands may have occurred by the movement of adults. As species of *Scomber* are pelagic and vagile, and comparisons among some distant locations (J-IVC and J-ARG; A-NZL and A-AUS) revealed no evidence of differentiation and was consistent with gene flow. Colonization of the Revillagigedo Islands by movement of adults appears to be a reasonable explanation.

Despite the widespread genetic similarity observed among samples of *S. australasicus*, exchange in the north/south direction is restricted. Genotypes of the unique New Zealand/Australia lineage were not observed in samples from Japan or Mexico. It would appear that the equatorial region of the Pacific is a barrier to gene flow in *S. australasicus*.

Genetic relationships among disjunct populations

Results of the present analysis demonstrated lower population structure than observed by RFLP analysis of the cosmopolitan gray mullet, *Mugil cephalis*. With sampling on a geographic scale similar to that in the present study, Crosetti et al. (1994) obtained values of divergence among samples of gray mullet that were similar to those observed among samples of *S. japonicus*; however, the number of genetically unique populations was greater in gray mullet. Contrasting patterns of relatedness were observed between gray mullet and *S. japonicus*, as fixed restriction site differences, and greater than 2% net nucleotide sequence divergence were demonstrated between gray mullet sampled the Mediterranean Sea, Mauritania, South Africa, and Florida. However,

lower nucleotide sequence divergence, 1.4%, was demonstrated between samples of gray mullet from Hawaii and the Galapagos Islands, indicating the possibility of greater exchange, or more recent isolation, among populations separated by the East Pacific Barrier, than among Atlantic samples.

The differences in population structure and richness between *Scomber* and gray mullet may relate to differences in historic times of dispersal, coupled with historically dynamic oceanographic conditions that facilitate or inhibit dispersal, and differences in larval stage durations. A difference in the potential for adult dispersal may explain the observed differences in population structure. While tagging studies have shown that both species are capable of long distance movements (Chang and Wu, 1977; Funicelli et al., 1989), mackerels are more adapted to the pelagic environment (as indicated by fusiform body style, high caudal fin aspect ratio, finlets, dorsalfin groove, etc.), possibly explaining the lower population richness in *Scomber*. But both species may have important adaptations for pelagic migration. Both gray mullet and *S. japonicus* are tolerant to environmental temperatures outside the ranges normally found within their distributions (Seckel and Yong, 1970; Schaefer, 1986), which has been interpreted in gray mullet as a thermal preadaptation which may account for the species' wide geographic distribution (Sylvester, 1974).

Studies of mackerel and mullet revealed a wide range of genotypic variability among intraspecific populations. Genotypic diversity and nucleotide sequence diversity ranged from h=0.0 to 0.83, and π=0.0 to 0.22% in gray mullet (Crosetti et al., 1994), and from h=0.60 to 0.95, and π=0.13 to 0.77% in *Scomber*. Similarly, in bluefish

(*Pomatomus saltatrix*), variability in an western Atlantic sample (N=372, h=0.70, π=1.23%) was considerably higher than a sample differing by 3 fixed restriction site differences from eastern Australia (N=19, h=0.10, π=0.07%), within which only one variant genotype was seen (Graves et al., 1992b). This contrasts considerably to the similarity observed in measures of variability among samples of yellowfin tuna collected from several geographically distant samples (Scoles and Graves, 1993 [previous chapter]). The wide differences in variability may indicate regional differences in historical processes leading to reduced population size (bottlenecks), with which the smaller effective population size of the mtDNA genome can be lead to a considerable loss of variability relative to nuclear DNA characters (Birky et al., 1983).

The level of nucleotide sequence divergence between Atlantic and Pacific samples of *S. japonicus* was low relative to their geographic isolation. It has been suggested that in mammals, mitochondrial DNA evolves at rate of 2% nucleotide sequence divergence per million years (Brown et al., 1979). Applying this rate of divergence to the 1.4% net nucleotide sequence divergence observed between Atlantic and Pacific *S. japonicus* suggested the two populations were isolated about 0.7 million years. 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, resulting from changing historical distributions, possibly driven by historical climatic changes (Avise, 1992), which might explain transgression of the tropical Pacific.

In several comparisons of *S. japonicus* and *S. australasicus*, the inability to reject the null hypothesis that sampled populations share a common gene pool is consistent with genetic exchange among populations. The homogeneity observed among some populations may be the result of high vagility in mackerels, and a lack of geographic and oceanographic barriers in the marine environment that might limit gene flow.

If mackerels of the genus *Scomber* have such a high propensity for gene flow, then what is responsible for the observed intraoceanic differentiation? In Pacific *S. japonicus*, it appears that isolation by distance may be the reason eastern and western samples possessed unique genotypes, as the lack of suitable habitat between sampled populations may limit exchange.

The results of this study also illustrate that physical oceanographic characteristics of the environment may play a significant role in shaping population pattern and richness. In west Atlantic *S. japonicus*, significant differentiation occurred across the equatorial region, and in *S. australasicus*, genotypes of the unique New Zealand/Australia lineage were not observed in samples above the equator. Similarly, a population-genetic study of the pelagic mako shark has demonstrated that mtDNA genotype frequencies were significantly different between samples from the western North and South Atlantic (Heist, 1994). 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 pelagic species.

The effects of oceanographic characteristics on population pattern and richness have been thoroughly reviewed and examined by Sinclair (1988), who concluded that

population richness 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. The data of the present study support the hypothesis that oceanographic characteristics are significant in limiting dispersal in *Scomber*, but also suggest that the exchange of adult individuals among populations may have a significant effect on the population-genetic structure in *Scomber*.

There have been few studies of genetic exchange among broadly distributed marine fishes with disjunct populations. In an electrophoretic analysis of 35 loci of 12 marine fish species collected from the Galapagos Islands, Gulf of California, and Panamá in the east Pacific, and from Hawaii in the central Pacific, Rosenblatt and Waples (1986) reported estimates of genetic distance similar to values often found among local populations ($D < 0.01$-0.06). These values were lower than estimates across the Isthmus of Panamá ($D = 0.13$-0.36; Vawter et al., 1980; Rosenblatt and Waples, 1986). The results indicated that the East Pacific Barrier is not a total barrier to genetic exchange, and it was suggested that the low estimates of divergence are the result of ongoing gene flow. But what would account for such widespread genetic homogeneity? In another allozyme analysis of 10 eastern Pacific shore fish species, Waples (1986) concluded that little genetic differentiation is likely to occur in marine fishes with high fecundities and pelagic larval life of 2 months or more unless physical or behavioral limitations of dispersal occur. Though it has been hypothesized that dispersal among widely distributed marine populations is a function of larval stage duration and adult size, Thresher and Brothers (1985) could find no such relationship among species or genera in angelfishes

(Pomacanthidae). Instead they suggested that geological features are better predictors of dispersal than adult size or larval stage duration in Indo-West Pacific pomacanthids, and that long distance dispersal perhaps occurs in only a few marine fish species with very long larval durations. The observed genetic homogeneity in *S. australasicus* between New Zealand and Australia, and in *S. japonicus* between Argentina and Ivory Coast, may support the latter supposition of Thresher and Brothers (1985), perhaps reflecting adaptations in *Scomber* for pelagic dispersal, either in early life history stages or adults.

DISCUSSION

The objective of this dissertation was to provide a better understanding of phylogenetic relationships and gene flow among populations of widely distributed marine fishes by comparing and contrasting the population-genetic structures of yellowfin tuna and mackerels of the genus *Scomber*. For each species, a single null hypothesis was tested: H_o: samples were collected from populations that share a common gene pool. The null hypothesis was rejected for all three species of *Scomber*, but not for yellowfin tuna. The data suggested that sufficient gene flow occurred among the sampled populations of yellowfin tuna to prevent divergence.

When the null hypothesis can not be rejected the result may be due to insufficient sampling. Pilot studies were conducted to ensure that the lack of differentiation observed in yellowfin tuna was not the result of insufficient sampling. It was demonstrated that for the characters analyzed, the apparent homogeneity was not an artifact of small sample sizes. Ward et al. (1994) further investigated yellowfin tuna population structure by RFLP analysis of mtDNA with larger sample sizes (n=434) and obtained the same result, substantiating the conclusion of the present study.

In contrast to yellowfin tuna, all three species of *Scomber* revealed significant intraspecific differentiation. However, a variety of divergence levels among populations was present; from homogeneity between samples, to the presence of fixed restriction site

differences between conspecific populations. Genetic homogeneity was evident among samples of *S. japonicus* from populations surrounding the Mediterranean and Atlantic coasts of Africa. In addition, homogeneity was observed between samples from Ivory Coast and Argentina, and between samples of *S. australasicus* from New Zealand and Australia--regions separated by potential thermal barriers or deep ocean basins. Like yellowfin tuna, the observed genetic similarity is consistent with recent genetic isolation, or exchange among populations facilitated by adult, or larval dispersal.

Significant intraspecific differentiation was observed within species of *Scomber* in other comparisons that suggested thermal barriers, or distance can limit gene flow. Significantly different distributions of mtDNA genotypes, or fixed restriction site differences, demonstrated the genetic isolation of *S. japonicus* from the Red Sea, California, and Florida. Unique genotypes in *S. australasicus* in New Zealand and Australia, not observed in Japan and Mexico, indicated that the equatorial Pacific is a barrier to gene flow.

What accounts for the observed differences in population structure between yellowfin tuna and species of *Scomber*? Because of large differences in body size, mackerel are not as vagile as yellowfin tuna and tagging studies demonstrated that yellowfin tuna have much greater dispersal than mackerel. Although few in number, tagging studies of *Scomber* revealed that movements are primarily restricted to within major regions. In *S. scombrus*, tagging indicated some evidence for exchange among the hypothesized North Sea and Western stocks, the spawning centers of which are separated by the English Channel, and nearly 1,000 km distance (Hamre, 1980). Release of 5,667

tagged *S. australasicus* off the north coast of Taiwan yielded 6 returns, three that were near the site of release, and three that were near the coast of Japan and Korea (Chang and Wu, 1977). Unlike mackerels, tagging of yellowfin tuna has shown that individuals are capable of moving thousands of kilometers between regions (Bayliff, 1984; Bard and Scott, 1991; Itano and Williams, 1992).

Adult and larval distributions of yellowfin tuna and mackerels also are known to be considerably different. Adults of mackerel are coastally distributed in populations separated by ocean basins, while adults of yellowfin tuna are continuously distributed in all tropical regions (Figs. 2-4, 6; Collette and Nauen, 1983). In mackerels, larvae are concentrated primarily near coastal regions (Hamre, 1980; Ware and Lambert, 1985; Sinclair et al., 1985; Sato, 1990). However, larvae of yellowfin tuna are continuously distributed throughout circumtropical waters (Nishikawa et al., 1985).

As predicted by the member/vagrant hypothesis of Sinclair (1988), population structure in mackerels of the genus *Scomber* is greater than population structure in yellowfin tuna. Differences in population structure between yellowfin tuna and species of *Scomber* appear to correspond to differences in numbers of larval retention areas, as indicated by the distribution of larvae. However, the data of the present study illustrate inconsistencies with the member/vagrant hypothesis. Spawning of yellowfin tuna occurs in equatorial regions that are not contiguous between the Pacific and Atlantic oceans (Nishikawa et al., 1985). The lack of significant differentiation between the Atlantic and Pacific yellowfin tuna apparently resulted from the movement of adults, as indicated by their appearance around the Cape of Good Hope in the southern summer (Talbot and

Penrith, 1962). Movement of adults through equatorial regions of the eastern Atlantic may also be significant in maintaining genetic homogeneity in *S. japonicus*.

Oceanographic processes are important in shaping population structure in marine fishes, illustrated in *S. japonicus* and *S. australasicus* by the apparent lack of trans-equatorial exchange in the Pacific and west Atlantic. However in the family Scombridae, biological characteristics that affect dispersal of both early-life history stages and adults appear to be important in the determination of species' population structure. This conclusion is strongly supported in yellowfin tuna by the lack of spawning indicated by low larval abundance in the region south of South Africa where in the southern summer adults are known to occur indicating the observed intraspecific homogeneity resulted from movement of adults through this region. In mackerels the conclusion is also supported by the observation of homogeneity among populations that are geographically distantly separated, that clearly indicated genetic exchange occurs among separate larval retention areas.

Appendix I. Restriction morphs of yellowfin tuna, *Thunnus albacares*, and restriction fragment sizes, in kilobase pairs.

ApaI						
A	B	C	D	E	F	G
—	—	—	—	—	7.8	—
7.1	—	—	7.1	7.1	—	7.1
—	—	5.4	—	—	—	—
—	4.8	—	—	—	—	—
—	—	—	—	4.7	—	—
4.0	4.0	4.0	4.0	—	4.0	4.0
—	—	—	3.5	—	—	—
3.0	3.0	3.0	—	3.0	3.0	—
—	—	—	—	—	—	2.8
—	2.3	—	—	—	—	—
—	—	1.7	—	—	—	—
0.7	0.7	0.7	0.7	0.7	0.7	0.7
0.7	0.7	0.7	0.7	—	—	0.7
0.6	0.6	0.6	0.6	0.6	0.6	0.6
0.5	0.5	0.5	—	0.5	0.5	0.5
—	—	—	—	—	—	0.2

AvaI
A
7.6
4.8
4.3

BanI		
A	B	C
—	—	8.2
5.0	—	—
—	4.4	—
3.3	3.3	3.3
3.2	3.2	—
2.6	2.6	2.6
1.9	1.9	1.9
—	0.6	—

BclI						
A	B	C	D	E	F	G
—	—	—	8.7	—	—	—
—	—	—	—	7.7	—	7.7
4.7	4.7	4.7	—	—	4.7	—
4.4	—	—	4.4	4.4	4.4	—
4.0	4.0	4.0	—	4.0	4.0	4.0
—	—	—	—	—	3.7	—
—	—	3.6	—	—	—	3.6
3.0	3.0	3.0	3.0	—	—	—
—	3.0	—	—	—	—	—
—	1.4	—	—	—	—	—
—	—	0.8	—	—	—	0.8
0.6	0.6	0.6	0.6	0.6	—	0.6

BglI				
A	B	C	D	E
—	—	10.9	—	—
8.5	8.5	—	8.5	8.5
—	—	—	5.4	—
3.0	3.0	3.0	—	3.0
—	—	—	—	2.8
2.4	—	—	—	2.4
—	2.1	—	—	—
1.5	1.5	1.5	1.5	—
1.3	1.3	1.3	1.3	—
—	0.3	—	—	—

DraI
A
7.8
5.0
3.2
0.3

EcoRI		
A	B	C
9.3	9.3	—
—	7.6	—
—	—	7.2
3.9	—	3.9
3.7	—	3.7
—	—	2.1

HindIII
A
5.1
3.8
3.0
1.6
1.4
1.2
0.6

NciI	
A	B
10.9	—
—	7.6
—	3.3
1.9	1.9
1.7	1.7
0.9	0.9
0.7	0.7

PstI	
A	B
—	7.9
5.3	—
5.0	5.0
3.6	3.6
2.6	—

PvuII	
A	B
—	10.4
8.8	—
6.5	6.5
1.6	—

XhoI
A
11.5
4.9

Appendix II. Restriction morphs of mackerels of the genus *Scomber*, and restriction fragment sizes, in kilobase pairs.

ApaI

A	B	C	D	E	G	H	I	J	K	L	M	N	O	P	R	S	V	W	X	Y	Z
—	—	—	—	—	—	—	8.5	8.5	—	8.5	8.5	—	8.5	8.5	—	—	—	—	—	8.5	—
—	—	—	—	—	—	—	—	—	8.5	—	—	—	—	—	—	—	—	—	—	—	—
7.0	7.0	7.0	7.0	—	7.0	7.0	—	7.0	—	—	—	—	—	—	—	—	—	—	—	—	—
7.0	—	—	—	—	—	7.0	—	—	—	—	—	7.0	—	—	7.0	7.0	7.0	7.0	7.0	—	7.0
—	—	6.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.0	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	5.2	—	—	—	—	—	—	—	—
—	—	—	—	5.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.0	5.0	—	—	—	—	—
—	5.0	—	5.0	—	—	—	—	—	5.0	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.6	—	—	—
—	—	—	—	3.8	3.8	—	3.8	—	—	3.8	3.8	3.8	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.5	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.3	3.3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.3	—	—	—	—
—	—	—	—	3.2	3.2	—	3.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	3.1	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.8	2.8	2.8	2.8	2.8
—	2.2	—	2.2	—	—	—	—	—	2.2	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.0	—	—	—	—	—	—
—	—	—	—	1.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	1.8	1.8	1.8	—	—	—	—	—	1.8	—	—
1.5	1.5	1.5	1.5	1.5	1.5	1.5	—	—	—	—	—	1.5	—	—	1.5	—	1.5	1.5	1.5	—	1.5
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.5	—	—
—	—	—	—	—	—	—	—	—	—	—	1.3	1.3	—	—	—	—	1.3	—	1.3	1.3	1.3
—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—	—	—	—	—	—
—	—	—	0.8	—	—	0.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.8	0.8	0.8	0.8	0.8
—	—	0.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.5	0.5	0.5	—	0.5	0.5	—	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	—	—	—	—	—
0.3	0.3	0.3	—	0.3	0.3	—	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	0.1	0.1	0.1	—	—	—	—	—	—	—	—	—

AvaI

A	B	C	D	E	F	G	H	Y	Z
—	9.8	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	7.2	7.2
6.5	—	6.5	—	6.5	6.5	—	6.5	—	—
—	—	—	—	—	—	—	—	4.7	—
—	—	—	—	—	—	—	—	—	4.5
—	—	—	—	—	—	—	4.4	—	—
—	—	—	4.1	—	—	—	—	—	—
—	—	—	—	—	—	3.6	—	—	—
3.3	—	—	3.3	3.3	—	3.3	3.3	—	—
—	—	—	—	—	—	2.9	—	—	—
2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
—	—	—	2.4	—	—	—	—	—	—
2.3	2.3	2.3	2.3	2.3	2.3	2.3	—	—	—
—	—	2.2	—	—	—	—	—	—	—
2.1	2.1	2.1	2.1	—	2.1	2.1	—	2.1	2.1
—	—	—	—	—	1.8	—	—	—	—
—	—	—	—	1.6	—	—	—	—	—
—	—	—	—	—	1.5	—	—	—	—
—	—	1.1	—	—	—	—	—	—	—
—	—	—	—	0.5	—	—	—	—	—
—	—	—	—	—	—	—	—	—	0.2

BglI

A	B	C	D	E	Z
—	—	—	—	—	14.0
6.8	6.8	6.8	6.8	6.8	—
6.8	—	—	6.8	6.8	—
—	—	3.9	—	—	—
—	3.8	—	—	—	—
3.3	3.3	—	—	—	—
—	—	3.1	—	3.1	—
—	3.0	—	—	—	—
—	—	2.9	—	—	—
—	—	—	—	—	2.9
—	—	—	1.8	—	—
—	—	—	1.5	—	—
—	—	0.2	—	0.2	—

Bsu36I

A	B	Z
—	—	16.5
8.0	8.0	—
4.9	4.9	—
—	3.6	—
2.8	—	—
0.8	—	—

DraI

A	B	C	D	E	X	Y	Z
—	—	9.6	9.6	—	—	—	—
—	—	—	—	—	8.5	—	—
8.4	8.4	—	—	—	—	—	—
—	—	—	—	7.7	—	—	—
—	6.0	—	—	—	—	6.0	6.0
—	—	—	—	—	—	4.8	—
—	—	—	—	—	4.2	—	4.2
3.0	—	3.0	—	3.0	—	—	—
3.0	—	3.0	3.0	3.0	—	—	—
—	—	—	—	—	—	2.5	2.5
—	—	—	2.5	—	—	—	—
—	—	—	—	—	1.5	1.5	1.5
1.2	1.2	—	—	1.2	1.2	1.2	1.2
—	—	—	—	0.7	—	—	—
0.6	0.6	0.6	0.6	0.6	0.6	—	0.6
—	—	—	0.5	—	—	—	—
—	—	—	—	—	0.4	0.4	0.4
—	—	—	—	—	0.3	0.3	0.3

HaeII

A	B	C	D	E	F	G	X	Y	Z
—	—	—	—	—	—	—	15.2	—	15.2
—	—	—	—	—	—	—	—	9.7	—
—	—	—	—	9.2	—	—	—	—	—
5.5	—	—	—	5.5	5.5	—	—	5.5	—
5.1	5.1	5.1	—	—	5.1	—	—	—	—
—	—	—	—	—	—	4.4	—	—	—
—	4.1	4.1	4.1	—	—	4.1	—	—	—
4.1	4.1	4.1	4.1	—	4.1	4.1	—	—	—
—	—	—	3.7	—	—	—	—	—	—
—	—	1.7	—	—	1.7	—	1.7	—	—
—	1.4	1.4	1.4	—	—	1.4	—	—	—
—	—	—	1.4	—	—	—	—	—	—
1.3	1.3	—	1.3	1.3	—	1.3	—	1.3	1.3
—	—	—	—	—	—	0.7	—	—	—
0.5	0.5	0.5	0.5	0.5	0.5	0.5	—	—	—
0.4	0.4	—	0.4	0.4	—	0.4	—	0.4	0.4

HpaI

A	B	C	D	E	F	Y
—	—	—	—	—	—	13.4
—	—	—	—	—	10.9	—
—	8.7	—	—	—	—	—
7.1	—	7.1	7.1	7.1	—	—
—	—	—	5.8	—	—	—
—	—	—	—	3.8	—	—
3.3	3.3	3.3	—	3.3	3.3	3.3
2.5	2.5	2.5	—	2.5	2.5	—
2.2	2.2	2.2	2.2	—	—	—
1.6	—	—	1.6	—	—	—
—	—	1.5	—	—	—	—
—	—	0.1	—	—	—	—

PvuII

A	B	Z
—	13.4	—
11.9	—	—
—	—	9.1
—	—	4.5
—	—	3.3
2.7	2.7	—
1.5	—	—
0.8	0.8	—

ScaI

A	B	C	X	Z
—	—	—	—	16.9
15.3	—	—	—	—
—	8.8	8.8	—	—
—	—	8.1	8.1	—
—	—	—	6.6	—
—	6.5	—	—	—
—	—	—	2.2	—
1.6	1.6	—	—	—

SpeI

A	B	C	Y	Z
—	16.1	—	—	—
9.5	—	—	—	—
6.6	—	6.6	—	—
—	—	—	5.7	5.7
—	—	5.6	—	—
—	—	—	—	5.1
—	—	—	4.3	—
—	—	3.9	—	—
—	—	—	2.0	2.0
—	—	—	1.9	1.9
—	—	—	1.6	1.6
—	—	—	0.8	—

SspI

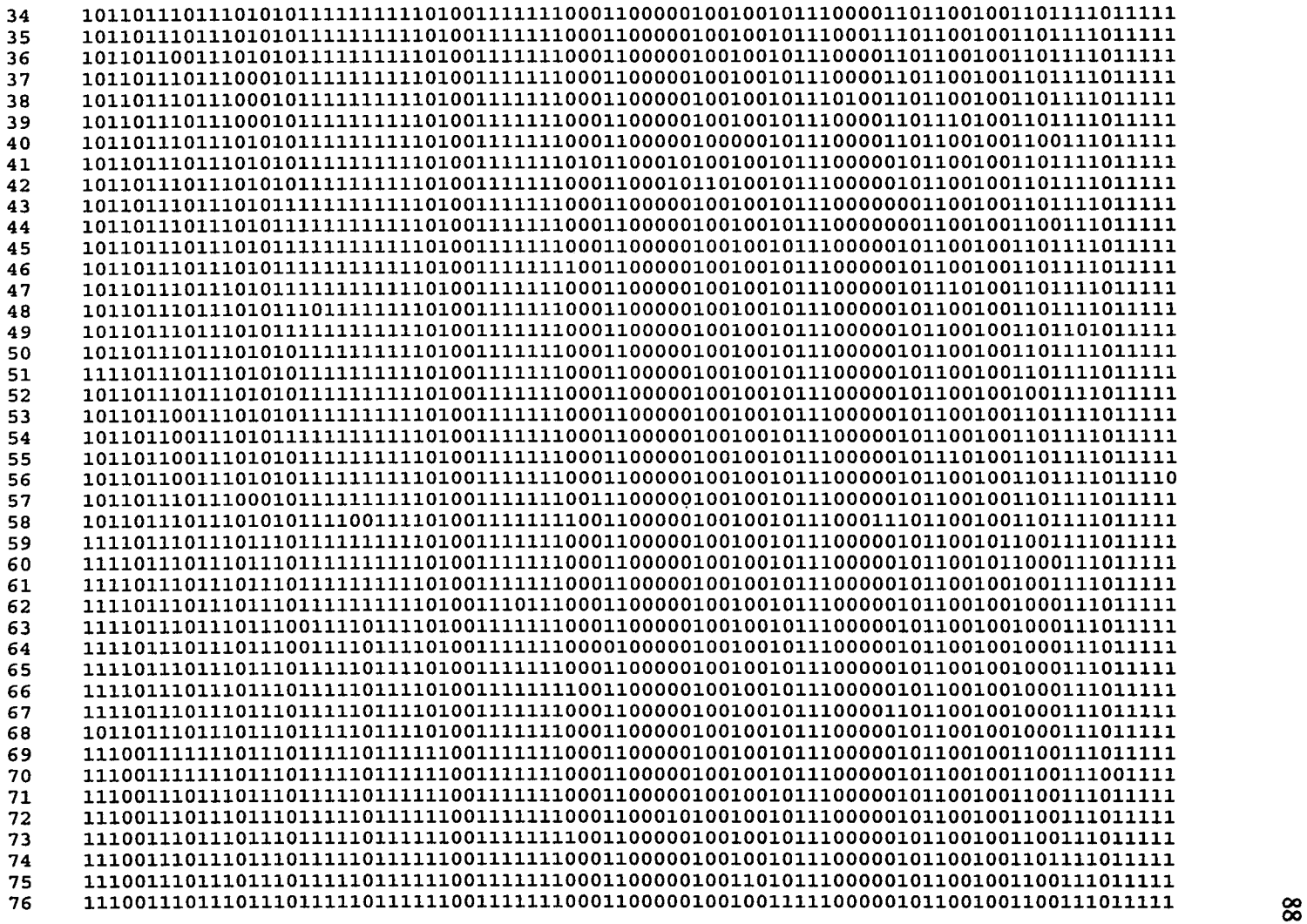
A	B	C	D	E	F	G	H	I	X	Y	Z
—	—	—	—	16.5	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	14.6	—	—	—
—	—	—	14.5	—	—	14.5	—	—	—	—	—
13.7	—	—	—	—	—	—	—	—	—	—	—
—	12.6	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	11.6	—	—	—	—
—	—	—	—	—	8.9	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	8.6	8.6	8.6
—	—	—	—	—	7.6	—	—	—	—	—	—
—	—	7.1	—	—	—	—	—	—	—	—	—
—	—	6.6	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	4.3	4.3	4.3
—	—	—	—	—	—	—	—	—	—	2.9	—
—	—	—	—	—	—	—	—	—	2.3	—	2.3
—	—	—	—	—	—	—	2.1	—	—	—	—
2.0	2.0	2.0	2.0	—	—	—	2.0	—	—	—	—
—	—	—	—	—	—	1.5	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1.1	—	—
—	1.1	—	—	—	—	—	—	1.1	—	—	—
0.8	0.8	0.8	—	—	—	—	0.8	0.8	—	—	—
—	—	—	—	—	—	—	—	—	—	0.8	0.8
—	—	—	—	—	—	—	—	—	0.6	—	0.6
—	—	—	—	—	—	0.5	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	0.3	0.3

StuI

A	B	C	D	E	F	G	H	I	J	K	L	Y	Z
—	8.5	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	7.3	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	4.8	4.8
4.7	—	4.7	4.7	4.7	—	4.7	4.7	4.7	4.7	—	4.7	4.7	4.7
3.8	—	3.8	—	—	—	3.8	3.8	—	—	—	—	—	—
—	—	3.2	—	—	—	—	—	3.2	3.2	—	—	—	—
—	—	—	—	—	—	—	—	—	—	3.1	—	—	—
—	—	—	—	—	—	—	—	—	—	—	2.6	—	—
—	—	—	2.6	2.6	—	—	—	2.6	2.6	2.6	2.6	—	—
—	—	—	—	—	—	—	—	—	—	—	—	2.5	—
—	—	—	—	2.0	2.0	—	2.0	—	—	2.0	—	—	2.0
1.9	1.9	—	1.9	1.9	1.9	1.9	1.9	—	—	1.9	1.9	—	—
1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	—	—
—	—	—	—	—	—	—	—	—	—	1.6	—	—	—
1.4	1.4	1.4	1.4	1.4	1.4	—	—	1.4	—	1.4	—	1.4	1.4
1.3	1.3	—	1.3	1.3	1.3	1.3	1.3	—	—	1.3	1.3	1.3	1.3
—	—	—	—	—	—	1.2	—	—	1.2	—	—	—	—
1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
—	—	—	1.2	—	—	—	—	1.2	1.2	—	—	—	—
0.8	0.8	0.8	0.8	—	—	0.8	—	0.8	0.8	—	0.8	—	—
—	—	—	—	—	—	—	—	—	—	—	—	0.6	0.6
—	—	—	—	—	—	—	—	—	—	—	—	—	0.5
—	—	—	—	—	—	0.2	0.2	—	0.2	—	—	—	—

Appendix III. Binary character matrix used in parsimony analysis of genotypes of *Scomber japonicus* and *S. australasicus*. The characters '1' and '0' denote the presence or absence of a restriction site for a particular genotype (left comumn number), as determined from hypothetical maps of restriction sites developed from the data in Appendix II. In the parsimony analysis, monomorphic characters were removed, although they are present here. Columns of characters determined from restriction enzymes are *Sca*I (1-3), *Dra*I (4-11), *Stu*I (12-22), *Pvu*II (23-26), *Hae*II (27-35), *Apa*I (36-48), *Ava*I (49-58), *Ssp*I (59-66), *Bgl*I (67-73), *Hpa*I (74-79), *Spe*I (80-82), and *Bsu*36I (83-86).

1	11110111011101110111101111111001111111000011011010010010111000001111001101100111011111
2	11110111011101110111101111111001111111000111011010010010111000001111001101100111011111
3	11110111011101110111101111111001111111000011011010010010111000001111001101100111111111
4	11110111011101110111101111111001111111000011011010010010011000001111001101100111011111
5	11110111011101110111101111111001111111000010011010010010111000001011001101100111011111
6	11110111011101110111101111111001111111000011011010010010111000001011001101100111011111
7	11110111011101110111101111111001111111000010001010010010111000001111001101100111011111
8	11110111011101110111101111111001111111000010011010010010111000001111001101100111011111
9	11110111011101110111101111111011111111000010001010010010111000001111001101100111011111
10	11110111011101110111101111111001111111000011000010010010111000000011001101100111011111
11	11110111011101110111101111111001111111000011000010010010111000001111001101100111011111
12	11110111011101110111101111111001111111000010000010010010111000001111001101100111011111
13	11110111011101110111101111111001111111000010000010010010111000001011001101100111011111
14	11110111011101110111101111111001111111000010000010010010111000001011001101101111011111
15	11110111011101110111101111111001111111000010001010010010111000001011001101101111011111
16	11110111011101110111101111111011111111000010001010010010111000001011001101101111011111
17	11110111011101110111101111111001111111000010001010010010111000001011001101101111011110
18	11110111011101110111101111111001111111000010001010110010111000001011001101101111011111
19	11110111011101100111101111111001111111000010001010010010111000001011011101101111011111
20	10110110011101010111111111110100111111111001100000100100101111000011011001001101111011111
21	10110110011101010111110111101001111111111001100000100100101111000011011001001101111011111
22	10110110011101010111111111110100011111111001100000100100101111000011011001001101111011111
23	10110110011101010111111111110100011111111001100000100100101111000001011001001101111011111
24	10110111011101010111111111110100111111111001100000100100101111000011011001001101111011111
25	10110110011101010111111111110100111111111001100000100100101111000011011001001101111011111
26	10110111011101010111111111110100111111111001100000100100101111000001011001001101111011111
27	10110110011101010111111111110100111111111001100000100100101111000001011001001101111011111
28	10110110011101010111111111110100111111011001100000100100101111000011011001001101111011111
29	10110110011101010111111111110100111111111001000000100100101111000001011001001101111011111
30	10110110011101010111111111110100111111111001100000100100101110000111011001001101111011111
31	10110111011101010111111111110100111111110000100000100100101111000011011001001101111011111
32	11110111011101010111111111110100111111110000100000100100101111000011011001001101111011111
33	10110111011101010111111111110100111111110000100001100100101111000011011001001101111011111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35 10110111011110101011111111110100111111100011000001001001011100001101100100110111110111111
36 10110110011101010111111111110100111111100011000001001001011100001101100100110111110111111
37 10110111011100010111111111110100111111100011000001001001011100001101100100110111110111111
38 10110111011100010111111111110100111111100011000001001001011101001101100100110111110111111
39 10110111011100010111111111110100111111100011000001001001011100001101110100110111110111111
40 10110111011110101011111111110100111111100011000001000001011100001101100100110011110111111
41 10110111011110101011111111110100111111101011000101001001011100000101100100110111110111111
42 10110111011110101011111111110100111111100011000101101001011100000101100100110111110111111
43 10110111011101010111111111110100111111100011000001001001011100000001100100110111110111111
44 10110111011110101111111111110100111111100011000001001001011100000001100100110011110111111
45 10110111011110101111111111110100111111100011000001001001011100000101100100110111110111111
46 10110111011110101111111111110100111111100110000010010010111000001011001001101111011111
47 10110111011110101111111111110100111111100011000001001001011100000101110100110111110111111
48 10110111011110101110111111110100111111100011000001001001011100000101100100110111110111111
49 10110111011110101111111111110100111111100011000001001001011100000101100100110111010111111
50 10110111011110101011111111110100111111100011000001001001011100000101100100110111110111111
51 11110111011110101011111111110100111111100011000001001001011100000101100100110111110111111
52 10110111011110101011111111110100111111100011000001001001011100000101100100100111110111111
53 10110110011110101011111111110100111111100011000001001001011100000101100100110111110111111
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55 10110110011110101011111111110100111111100011000001001001011100000101110100110111110111111
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62 11110111011101110111111111110100111011100011000001001001011100000101100100100011110111111
63 11110111011101110011110111101001111111100011000001001001011100000101100100100011110111111
64 11110111011101110011110111101001111111100001000001001001011100000101100100100011110111111
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66 11110111011101110111101111010011111111100110000010010010111000001011001001000111011111
67 11110111011101110111101111010011111111100011000001001001011100001101100100100011110111111
68 10110111011101110111101111010011111111100011000001001001011100000101100100100011110111111
69 11100111111101110111101111010011111111100011000001001001011100000101100100110011110111111
70 11100111111101110111101111010011111111100011000001001001011100000101100100110011100111111
71 11100111011101110111101111010011111111100011000001001001011100000101100100110011110111111
72 11100111011101110111101111010011111111100011000101001001011100000101100100110011110111111
73 11100111011101110111101111010011111111100110000010010010111000001011001001100111011111
74 11100111011101110111101111010011111111100011000001001001011100000101100100110111110111111
75 11100111011101110111101111010011111111100011000001001101011100000101100100110011110111111
76 11100111011101110111101111010011111111100011000001001001111100000101100100110011110111111

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77    11100111011101110111110111111001111101000110000010010010111000001011001001100111011111
78    11100111011101110111110111111001110111000110000010010010111000001011001001100111011111
79    11110111011001110101111111111100111111110001100000100101101110000000110010011111111011111
80    11110111011100110101111111111100111111110001100000100101101110000000110010011111111011111
81    11110111011001110101111111111100111111110001100000100101101110010000110010011100111011111
82    11010111011001110101111111111100111111110001100000100101101110010000110010011100111011111
83    11110110011001110101111111111100111111110001100000100101101110010000110010011100111011111
84    11110111011001110101110111111100111111110001100000100101101110010000110010011100111011111
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89    11110111011001110101111111111100111111110001100000100000101110010000110010011100111011111
90    11110110011001110101110111111100111111110001101000100101101110010000110010011100111011111
91    11110111011111111010111111111100111111110001100000100101101110010000110010011100111011111
92    11111111011111111010111011111100111111110001100000100101101110010000110010011100111011111
93    11110110011101110111111111111100111111110001100000100101101110010000110010011100111011111
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Appendix IV. DNA sequences of cytochrome *b* of 5 species of *Scomber*, and *Rastrelliger kanagurta* amplified using primers L15079 and H15479 from Finnerty and Block (1992). Reported sequences of *S. japonicus* from Italy and *S. scombrus* are from Finnerty and Block (1992). The speciman of *S. japonicus* from California had the common mtDNA genotype #15. The speciman of *S. australasicus* from Australia had the common mtDNA genotype #71 and was from the "unique" lineage of genotypes found only in New Zealand and Australia. A '.' represents missing data.

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------------------------------------------------------------------------
                    primer L15079-gaggcctctactatggctcttacc-3'
1-Rastrelliger                5'-gaggcctctactatggctcttacc tctatataga
2-S. scombrus                    gaggcctgtactacggctcatacc tttttgtaga
3-S. japonicus, Italy            gaggtctttactacggttcctacc tttttatgga
4-S. japonicus, California       gaggtctttactacggttcctacc ttttcatgga
5-S. australasicus, Australia    gaggcctctactatggctcttacc ttttcatgga

   20         30         40         50         60         70
1- aacatgaaac atcggagttg ttcttctcct cctagtaatg ataaccgctt tcgttggcta
2- gacatgaaac gtcggtgtag tcctcctcct cctcgtaatg atgactgcct tcgttggcta
3- gacatgaaac attggtgtgg ttcttctcct cctcgtaata ataaccgcat tcgtcggcta
4- aacatgaaac attggtgtgg ttcttctcct cctcgtaata ataaccgcat tcgtcggcta
5- aacatgaaac attggtgtag ttcttctcct cctcgtaata ataaccgcat tcgtcggcta

   80         90         100        110        120        130
1- cgtccttccc tgaggacaaa tgtccttctg aggcgcaact gtcattacta acctcctttc
2- cgtccttccc tgaggacaaa tgtccttctg aggagccact gtcatcacta acctactctc
3- cgtccttccc tgaggtcaaa tgtccttctg aggggccact gtcattacga acctactctc
4- c.tccttccc tgaggccaaa tgtccttctg aggggccacc gtcattacaa atctactttc
5- cgtccttccc tgaggccaaa tgtccttctg aggggccacc gtcattacaa acctactttc

   140        150        160        170        180        190
1- cgcagtcccc tatgtaggca ctaccctagt agaatgaatc tgaggtggct tctcctgcga
2- agcagttcca tacgtaggca caacactcgt tgaatgaatt tgagggggct tctcagtaga
3- ggcagtccca tacgttggta ctacactcgt tgagtgaatc tgaggcggct tctcagtaga
4- agcagtccca tac.tcggta ctacc.tcgt tgagtgaatc tgaggc.gct tctcagtaga
5- ggcagtccca tacgtcggta ctacactcgt tgagtgaatc tggggcggct tctcagtaga

   200        210        220        230        240        250
1- caatgcaacc ctcactcgat tcttcgc... .......... .......... ..........
2- caatgctacg ctcactcggt tcttcgcctt tcacttccta ttccccttg ttatcttagc
3- caatgccacc ctcacccgat tctttgcctt ccacttccta ttcccattcg tcatcctggc
4- caatt.cacc ctcacccgat tctttgcctt ccacttccta ttcccattcg ttatcctggc
5- caatgccacc ctcacccgat tctttgcctt ccacttccta ttcccattcg ttatc.tagc

   260        270        280        290        300        310
1- .......... .......... .......... .......... .......... ..........
2- ggcagcggtt ctccacctac tattccttca tgaaactggg tcaaacaacc caattggcct
3- agcaacaatt cttcacctgc tattcctaca tgaaaccggg tcaaacaacc caattggcct
4- agcaacaatt cttcacctgc tattcctaca tgagaccggg tcaaacaacc caattggcct
5- ggcaacaatt cttcacctgc tattcctaca tgaaaccggg tcgaacaacc caattggcct

   320        330        340        350        360        370
1- .......... ....ataaaa tctccttcct cccctact.c acctacaaat atg.cctagg
2- aaactctaat gcagacaaaa tctcgttcca tccgtacttt acgtataaag acctcctcgg
3- aaactctaat gcagacaaaa tctccttcca cccatacttc acctacaaag acctccttgg
4- aaactccaat gcagacaaga tctccttcca cccatacttc acctacaaag acctcctt.g
5- aaactccaat gcggacaaaa tctccttcca cccatacttc acctacaaag acctcctt.g
```

```
   380        390        400        410        420
1- atttgccatc cttcttatgg ctctcacatc cctagcactc ttctcc...a
2- ctttgccgtt ctccttatag gccttacctc cctagcactc ttctccccga
3- ctttgccgtc ctcctcgtag ccctttcctc tctagcactc t.........
4- ctttgccgtc ctcctcgtgg ccctctcctc t.tcgcactc ttctctccca
5- ctttgccgtc cttctcgtgg ccctctcctc tctcgcactc ttccctccca

  430     437
1-acctctt ggcgacccagacaattataccctagc-3'
2-acc.... ..........................
3-.......  ..........................
4-acctctt ggcgacccagacaattataccctagc
5-acctcct ggcgacccagacaattataccctagc
       5'-ggcgacccagacaattataccctagc-primer H15479
```

Appendix V. Cytochrome *b* DNA sequences of Appendix IV with primer sequences and all characters with missing values removed, resulting in 77 informative characters.

1-*Rastrelliger* cataaaacattagacttacatttcctccctactcaaaca
2-*S. scombrus* tttgaggctaccggtctaactctcacatacacaactata
3-*S. japonicus*, Italy tttaggattgtcaacactgcttgcacgcactttactgca
4-*S. japonicus*, California ttcagaattgtcaacaccgcctatatacaccttcctgca
5-*S. australasicus*, Australia ttcagaattatcaacaccgcctacatgcaccttactgcg

1- tctgcgactactactccccttgaaacttagctcaaca
2- gagtagtgtgccagatgtgtgccccgtctaagctacca
3- cagtagcccatcacacacccgcctcgcccgaccttcta
4- cgcacacccgcctcgcccggccctctccagtatcccat
5- cacacacccgcctcgctcggccctctccagtagcccat

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