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Mitochondrial DNA analyses of narrow-barred Spanish mackerel (*Scomberomorus commerson*) suggest a single genetic stock in the ROPME sea area (Arabian Gulf, Gulf of Oman, and Arabian Sea)

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We studied the genetic stock structure of *Scomberomorus commerson* (locally called kingfish) using restriction fragment length polymorphism (RFLP) and direct sequencing analyses of mtDNA samples from seven locations within the ROPME sea area (Arabian Gulf, Gulf of Oman, and Arabian Sea). A 475-bp segment from the D-loop region was screened in 218 samples using six restriction enzymes, resulting in 22 composite haplotypes. Mean nucleotide diversity for the seven populations was 0.025 (±0.000). An AMOVA comparison among groups of individuals inside and outside the Gulf showed 0.49% variation ($p = 0.201$), whereas the variation of populations within these groups was 0.05% ($p = 0.408$). The mean F_{ST} value for population pairwise comparisons was 0.010. To further resolve genealogies, sequence analysis was performed on a 330-bp fragment from the same segment for 193 fish. The genetic variance estimated across all populations was similar to the RFLP data, indicating a homogeneous distribution consistent with a single intermingling genetic stock. Based on the genetic marker tested, the null hypothesis that kingfish within the ROPME sea area constitutes a single stock cannot be rejected, but considering that a few migrating fish can reduce heterogeneity to where genetic drift is undetectable, panmixia cannot be confirmed. The results cautiously suggest that adopting a single-stock model and regional shared management are appropriate for sustainable long-term use of this important resource. More rigorous genetic testing using additional neutral markers, and mark-recapture experiments to detect spatial movement patterns, are recommended to further elucidate any stock substructure.

Keywords: mitochondrial DNA, narrow-barred Spanish mackerel, ROPME sea area, *Scomberomorus commerson*, stock structure.

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Introduction

The narrow-barred Spanish mackerel *Scomberomorus commerson* (Lacépède, 1800) is an epipelagic predator found throughout the Indo-West Pacific, including contiguous distribution within the ROPME (Regional Organization for the Protection of the Marine Environment) sea area (Figure 1; Al-Abdessalaam, 1995; Assadi and Dehghani, 1997; Carpenter *et al.*, 1997). ROPME encompasses an area including the Arabian Gulf (also known as the Persian Gulf, hereafter referred to as Gulf), the Gulf of Oman and the Arabian Sea; and, includes member states Bahrain, Iran, Iraq, Kuwait, Oman, Qatar, Saudi Arabia, and the United Arab Emirates (UAE).

Recognized locally as chanaad (Arabic) and kingfish (English), *Scomberomorus commerson* is one of the most important and highly valued commercial species exploited in the ROPME sea area, supporting substantial artisanal

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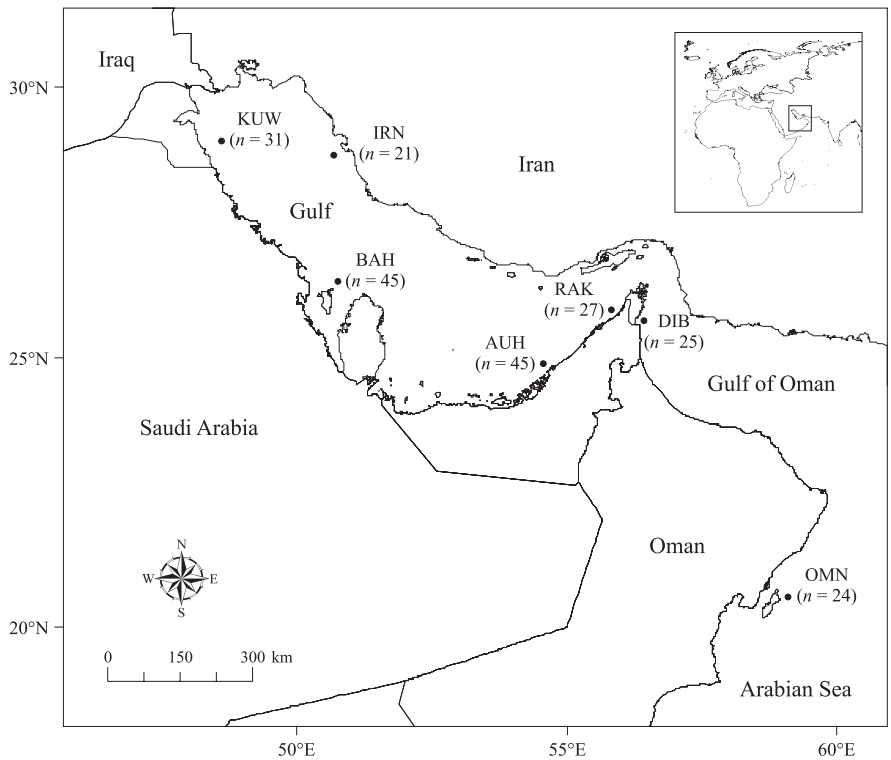

Figure 1. ROPME sea area, i.e. the Arabian Gulf, the Gulf of Oman, and the Arabian Sea, showing seven kingfish sampling locations: Abu Dhabi, UAE (AUH); Bahrain (BAH); Kuwait (KUW); Bushehr, Iran (IRN); Ras Al Khaimah, UAE (RAK); Dibba, UAE (DIB); and Masirah, Oman (OMN). RFLP sample sizes are shown in parentheses. A subset of samples was used for sequence analysis (see Table 2).

gillnet and handline fisheries (Al-Hosni and Siddeek, 1999). Classed as a neritic species, kingfish live predominantly in shallow coastal waters associated with continental shelves, along which the adults undertake extensive seasonal longshore migrations (Collette and Russo, 1984). ROPME sea area kingfish undergo rapid growth, reaching 80 cm fork length and maturity by two years of age (Dudley *et al.*, 1992; Al-Oufi *et al.*, 2004; Grandcourt *et al.*, 2005).

Currently, management of kingfish is administered individually by the eight nations within the ROPME sea area, although the region is comparatively small, and the incidence of straddling stocks and transboundary movements are believed to be extensive. Moreover, there is a lack of information on movements, migrations, and spawning activities to describe the stock structure of kingfish in the area.

Understanding fish stock structure is an important component of successful and sustainable long-term management (Shaklee and Currens, 2003), and molecular genetic techniques offer the ability to identify and delineate fish stock structure where it may not be apparent from phenotypic or behavioural characteristics (Magoulas, 2005). Such techniques have been used successfully to understand the structure of *Scomberomorus* spp. elsewhere (Gold *et al.*, 1997; Buonaccorsi *et al.*, 1999; Ovenden and Street, in press).

The Gulf is positioned northwest of the Gulf of Oman (Figure 1) and is connected by a single narrow passage at the Strait of Hormuz (Reynolds, 1993). Interestingly, sailfish (*Istiophorus platypterus*), another large highly vagile predator of the region, is apparently separated biogeographically at the Strait of Hormuz, and exhibits significant genetic substructure and very little mixing between populations inside and outside the Gulf (Hoolihan *et al.*, 2004). The geographical distribution of sailfish and kingfish in the ROPME sea area coincides, but whether the genetic structure of the kingfish population inside the Gulf exhibits a similar pattern of divergence remains to be tested rigorously. Area fishers contend that kingfish undertake seasonal migrations into and out of the Gulf, but no mark-recapture or other empirical evidence is available to substantiate such claims.

Kingfish have been heavily exploited in the ROPME sea area, raising concerns of possible growth-overfishing and recruitment failure (Dudley *et al.*, 1992). In the UAE, for example, the estimated rate of annual instantaneous fishing mortality (F) is 0.62 year^{-1}, a level considerably above the recommended target ($F_{opt} = 0.13$ year^{-1}) and limit ($F_{limit} = 0.17$ year^{-1}) biological reference points (Grandcourt *et al.*, 2005), clearly underscoring the importance of understanding stock structure for improved management.

Our objective for this study was to employ molecular techniques to determine whether ROPME sea area kingfish form a single stock, or are genetically subdivided into distinctly separate populations, by testing the null hypothesis of a single panmictic stock presence. For the purpose of definition, we refer to the biological description of a stock as given by Ihssen *et al.* (1981) to mean: "a stock is an intraspecific group of randomly mating individuals with temporal and spatial integrity". For these experiments, we chose to analyse mtDNA because its gene flow and hybridization zones are particularly well-suited to identifying population structure (Avise, 1994). Restriction fragment length polymorphism (RFLP) and sequence analyses were used to scrutinize the non-coding control region (D-loop) of the mtDNA molecule, because it is characteristically hypervariable and often exhibits substantial intraspecific polymorphism (Billington, 2003; Magoulas, 2005).

Material and methods

DNA extraction

Tissue samples from dorsal fin clips were collected from 218 kingfish >50 cm fork length (L_F) between December 2001 and October 2002 from seven locations in the Gulf, Gulf of Oman, and Arabian Sea (Figure 1). Sample locations (identification abbreviation and number of fish included) are the following: Abu Dhabi, UAE (AUH, 45); Bahrain (BAH, 45); Kuwait (KUW, 31); Bushehr, Iran (IRN, 21); Ras al Khaimah, UAE (RAK, 27); Masirah, Oman (OMN, 24); and Dibba, UAE (DIB, 25). Fin clip samples were stored in DMSO buffer medium (Seutin *et al.*, 1991) at 4°C until subjected to DNA extraction using DNeasy™ tissue kits (Qiagen GmbH, Germany). In turn, 25 mg quantities were digested with 40 µl of (20 mg ml^{-1}) proteinase K at 55°C while incubated overnight. Genomic DNA extraction was carried out as per the manufacturer's instructions.

Amplification and detection

The primers used for mtDNA amplification of a ~475 base pair (bp) segment were Pro889U20 (5′CCW CTA ACT CCC AAA GCT AG3′) and TDKD1291L21 (5′CCT GAA ATA GGA ACC AAA TGC3′), as described by Ovenden and Street (in press), and processed using Ready-to-go PCR beads (Amersham Biosciences, Uppsala, Sweden) containing 10 mM Tris−HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each oligonucleotide, 1.25 units of Taq DNA polymerase, and 20 ng of template DNA in a 25.0 µl reaction. Amplification was performed in a Progene (Techne Cambridge Ltd) thermal cycler programmed for an initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 15 s, 55°C for 24 s, 72°C for 30 s, and a final extension of 72°C for 5 min. Then 5 µl of the amplified products were subjected to 1% agarose/1% synergel gel electrophoresis for 45 min, stained with ethidium bromide, and visualized by UV transilluminator.

RFLP analysis

A total of 27 restriction enzymes was screened for 20 individuals. Six restriction enzymes exhibiting polymorphism in the preliminary analysis were further tested on all 218 samples; these included *Alu*I, *Dde*I, *Nla*III, *Nsp*I, *Tsp*509I (New England Biolabs®), and *Afa*I (Amersham Biosciences®). Restriction digestions included 3.2 µl PCR product, 0.4 µl restriction enzyme, and 0.4 µl buffer, and were carried out in a water bath at 37°C for 75 min for all enzymes except *Tsp*509I, which was incubated at 65°C for 75 min. Restriction fragments were separated in 12.5% precast polyacrylamide gel (Amersham Biosciences®) at 600 V, 25 mA, 15 W at 15°C, and visualized after silver staining (Amersham Biosciences®), as per the example in Figure 2. All enzyme profiles are available on request.

DNA sequencing

Amplified PCR products were purified using Qia quick PCR purification kits (Qiagen GmbH, Germany), as recommended by the manufacturer. The primers employed for PCR amplification were used to determine the sequences on both strands using the DNA cycle sequencing reaction kit protocol recommended by the supplier. The DNA sequences were obtained with an ABI 310 automated sequencer using the chain-termination method with big-dye terminators (Applied Biosystems, Foster City, CA). Forward and reverse sequences of automated base calls were visually examined on electropherograms, and any ambiguous base call conflicts were resolved, or excluded, by comparison and alignment using the program SeqScape® v 2.5 (Applied Biosystems, Foster City, CA). This produced a subsample of 193 kingfish, using a 334-bp portion of

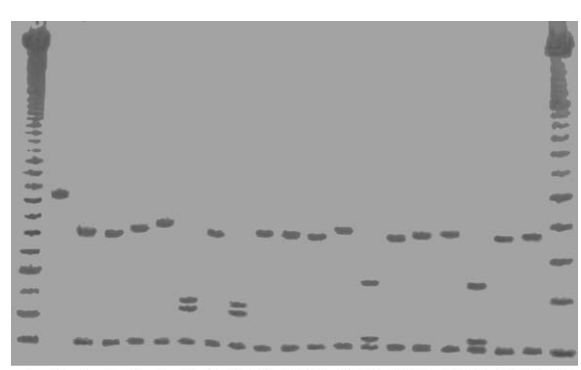

Figure 2. An example of polyacrylamide restriction fragment profile for the *Alu*I enzyme. From left, lane 1 contains 100 bp ladder, lane 2 contains uncut sample, lanes 3, 7, and 14 represent three haplotype variants for *Alu*I, and the lane on the extreme right contains 50 bp ladder.

the original 475 bp fragment, for further analysis. Sequences are available from the European Molecular Biology Laboratory database (EMBL) under the accession numbers AM234345−AM234537.

Data analyses

A character state matrix showing the presence or absence of putative restriction sites created in program Arlequin ver. 2.0 (Schneider *et al.*, 2000) was used to construct 22 operational taxonomic units, or composite haplotypes, from RFLP data, and program DnaSP ver. 4.1 (Rozas *et al.*, 2003) was used to construct 116 haplotypes from sequence data for further analysis in Arlequin. Population pairwise comparisons using haplotype frequencies were used to calculate analysis of molecular variance (AMOVA) and conventional F statistics (F_{ST}).

Nucleotide diversity (π) within populations and nucleotide divergence (d_{xy}) among populations (Nei and Tajima, 1981) were estimated using the D and DA modules in program REAP (McElroy *et al.*, 1992) for RFLP data, and in program DnaSP (Rozas *et al.*, 2003) for sequence data. An exact test of population differentiation for RFLP and sequence data using 10 000 Markov steps was calculated, as described by Raymond and Rousset (1995) and Goudet *et al.* (1996). Analyses were conducted between each of the seven populations and between two groups formed by the populations inside and outside the Gulf. The correlation between F_{ST} and geographical distance between populations was assessed with the Mantel test statistic Z (Mantel, 1967), calculated in program TFPGA (Miller, 1997), whereby the distance between each population pair was estimated as the shortest reasonable straight-line swimming route linking locations.

A minimum spanning tree of the hierarchical clustering relationships among pairwise distances between 116 sequence haplotypes for all populations (Rohlf, 1973) was constructed in Arlequin and illustrated with CorelDraw ver. 11.6 (Corel Corp., Canada).

Results

RFLP analysis of 218 kingfish exhibited 14 putative restriction sites from six polymorphic enzymes, resulting in 22 composite haplotypes. Just five RFLP haplotypes (h1, h6, h7, h15, and h18) account for 192 (88%) of the 218 fish (Table 1). Alternatively, direct sequence analysis of 308 usable nucleotide sites for 193 kingfish produced 116 haplotypes. The sequence transition/transversion ratio was 4.17, while the nucleotide composition exhibited an AT-bias ($A = 38.01\%$, $C = 21.09\%$, $G = 8.75\%$, $T = 32.15\%$).

Table 1. Distribution of 22 composite mtDNA haplotypes from RFLP data. Letters reflect individual haplotypes for restriction enzymes *Afa*I, *Alu*I, *Dde*I, *Nla*III, *Nsp*I, and *Tsp*509I (left to right).

		Inside Gulf						Outside Gulf			
Number	Composite haplotype	AUH	BAH	KUW	IRN	RAK	Total inside	DIB	OMN	Total outside	Total all
h1	AAAAAA	13	7	4	5	3	32	2	4	6	38
h2	AAAAAC	0	0	0	0	1	1	0	0	0	1
h3	AAAABA	0	0	0	0	0	0	0	1	1	1
h4	AABAAA	0	1	0	0	0	1	0	0	0	1
h5	ABAAAA	0	1	0	0	0	1	0	0	0	1
h6	BAAAAA	22	25	19	9	13	88	18	13	31	119
h7	BAAAAB	2	3	2	0	4	11	2	0	2	13
h8	BAAAAC	1	1	0	1	0	3	0	0	0	3
h9	BAAABA	1	0	0	0	0	1	0	0	0	1
h10	BAAABB	0	0	0	0	0	0	0	1	1	1
h11	BAABAA	0	0	0	0	1	1	0	0	0	1
h12	BAABAB	1	0	0	1	0	2	0	1	1	3
h13	BAABBA	0	0	0	0	1	1	0	0	0	1
h14	BAACAA	0	0	0	0	0	0	1	1	2	2
h15	BABAAA	3	3	1	3	2	12	1	0	1	13
h16	BABDAA	0	0	0	0	0	0	1	0	1	1
h17	BACAAA	1	1	0	1	0	3	0	0	0	3
h18	BBAAAA	0	2	4	0	1	7	0	2	2	9
h19	BCAAAA	1	0	0	1	0	2	0	1	1	3
h20	BCAAAB	0	0	0	0	1	1	0	0	0	1
h21	BDAAAA	0	0	1	0	0	1	0	0	0	1
h22	CAAAAA	0	1	0	0	0	1	0	0	0	1
Total		45	45	31	21	27	169	25	24	49	218

In all, 18 RFLP haplotypes were present inside the Gulf ($n = 169$) and 11 outside ($n = 49$; Table 1), whereas a total of 98 sequence haplotypes was present inside the Gulf ($n = 147$) and 32 outside ($n = 46$).

Genetic diversity within populations

Estimates of nucleotide diversity (π) derived from RFLP data for the seven populations ranged from 0.016 (DIB) to 0.030 (IRN, RAK, OMN), and populations pooled into two groups (inside and outside the Gulf) had nearly equivalent nucleotide diversities of 0.025 and 0.023, respectively (Table 2). In comparison, π estimates for the sequence data ranged from 0.035 (BAH, DIB) to 0.042 (KUW, RAK), while the pooled groups inside and outside the Gulf were 0.038 and 0.036, respectively. The highest numbers of observed haplotypes per population were 10 for BAH (RFLP data) and 33 for AUH (sequence data). The ratios (n_h/n_i) for the number of haplotypes (n_h) to the number of fish sampled for each population (n_i) ranged from 0.194 (KUW) to 0.333 (IRN, RAK, OMN) for RFLP data, and from 0.760 (DIB) to 0.941 (IRN) for sequence data (Table 2).

Genetic diversity among populations

The levels of nucleotide diversity among populations were similar to those within populations. Mean π values for pairwise comparisons among the seven populations were 0.025 (RFLP) and 0.037 (sequence), and 0.024 (RFLP) and 0.037 (sequence) among groups inside and outside the Gulf, respectively (Table 2). For the RFLP data, each population shared haplotypes with the other six. Low F_{ST} values for RFLP (<0.065) and sequence data (<0.036) were observed for all pairwise comparisons, but five comparisons (two RFLP and three sequence) exhibited significant values of p (Table 3). Following sequential Bonferroni correction (Rice, 1989), only the DIB:KUW pairwise comparison for the sequence analysis exhibited a significant difference.

The low F_{ST} values observed in the RFLP data were reflected in the exact test of population differentiation (non-differentiation exact p values), which showed no significant differences (data not shown) among populations or groups inside and outside the Gulf. For the sequence data, the significant non-differentiation exact p values observed (data not shown) were nearly consistent with the significant p values for F_{ST}. The one exception was the AUH:KUW comparison, for which the non-differentiation exact p value was significant ($p < 0.05$), and the corresponding F_{ST} p value (0.054) was slightly non-significant. In comparison, F_{ST} values between two groups formed by pooling populations inside and outside Gulf were 0.005 ($p = 0.198$) for RFLP data and 0.008 ($p < 0.01$) for sequence data.

AMOVA hierarchical analysis showed that most of the variation in haplotype frequencies could be attributed to variance within groups (RFLP 99.47%, $p \geq 0.322$; sequence 99.04%, $p \geq 0.062$), followed by variation among groups (RFLP 0.49%, $p \leq 0.202$; sequence 0.74%, $p \leq 0.045$), and finally, variation among populations (RFLP 0.05%, $p \leq 0.452$; sequence 0.22%, $p \leq 0.056$). There was no evidence of isolation by distance as pairwise F_{ST} and geographical separation between all pairs of locations sampled in the total data set using the Mantel test ($Z = 108.4$, $p = 0.850$), or among the five populations inside the Gulf ($Z = 13.0$, $p = 1.000$).

The minimum spanning tree constructed of 116 sequence haplotype relationships for all populations, portrayed as a bubble diagram (Figure 3), showed no evidence of spatial heterogeneity or clustering among the seven populations or between the two groups inside and outside the Gulf.

Discussion

Both the mtDNA RFLP and direct sequencing data exhibited very little divergence of haplotype frequencies

Table 2. Estimates of genetic diversity within populations for RFLP and sequence (SEQ) data: number of haplotypes (n_h), the ratio of n_h to the number of individuals sampled, n_i:n_h/n_i, haplotype diversity (h), and nucleotide diversity (π) within each of seven populations, grouped by region (inside and outside the Gulf) for kingfish sampled from locations in the ROPME sea area.

	n_i		n_h		n_h/n_i		$h \pm$ s.d.		π	
Population	RFLP	SEQ	RFLP	SEQ	RFLP	SEQ	RFLP	SEQ	RFLP	SEQ
AUH	45	42	9	33	0.200	0.786	0.684 ± 0.055	0.986 ± 0.009	0.025	0.038
BAH	45	38	10	30	0.222	0.789	0.669 ± 0.071	0.982 ± 0.012	0.024	0.035
KUW	31	24	6	19	0.194	0.792	0.604 ± 0.091	0.982 ± 0.016	0.019	0.042
IRN	21	17	7	16	0.333	0.941	0.767 ± 0.071	0.993 ± 0.023	0.030	0.041
RAK	27	26	9	24	0.333	0.923	0.749 ± 0.079	0.991 ± 0.015	0.030	0.042
DIB	25	25	6	19	0.240	0.760	0.483 ± 0.119	0.960 ± 0.029	0.016	0.035
OMN	24	21	8	17	0.333	0.810	0.692 ± 0.095	0.967 ± 0.030	0.030	0.038
Inside Gulf	169	147	18	98	0.107	0.666	0.685 ± 0.033	0.986 ± 0.004	0.025	0.038
Outside Gulf	49	46	11	32	0.224	0.696	0.589 ± 0.080	0.942 ± 0.028	0.023	0.036

Table 3. Population pairwise F_{ST} values for mtDNA RFLP data *below diagonal* and sequence data *above diagonal*. Following sequential Bonferroni correction, only the DIB:KUW sequence comparison showed a significant difference.

	AUH	BAH	KUW	IRN	RAK	DIB	OMN
AUH		0.000^{ns}	0.007^{ns}	0.006^{ns}	-0.002^{ns}	0.013^{ns}	0.000^{ns}
BAH	-0.002^{ns}		0.006^{ns}	0.002^{ns}	-0.004^{ns}	-0.003^{ns}	0.001^{ns}
KUW	0.020^{ns}	-0.015^{ns}		0.003^{ns}	0.006^{ns}	0.035***	0.016*
IRN	-0.023^{ns}	-0.005^{ns}	0.026^{ns}		0.002^{ns}	0.036*	0.012^{ns}
RAK	0.007^{ns}	-0.014^{ns}	-0.039^{ns}	-0.002^{ns}		0.011^{ns}	-0.005^{ns}
DIB	0.052*	0.003^{ns}	-0.004^{ns}	0.065*	0.020^{ns}		0.002^{ns}
OMN	-0.039^{ns}	-0.016^{ns}	-0.019^{ns}	-0.007^{ns}	-0.004^{ns}	0.013^{ns}	

^{ns}not significant; *$p < 0.05$; ***$p < 0.001$.

among the seven populations and two groups analysed, suggesting admixture within the ROPME sea area. Estimates of gene flow using F_{ST}, which expresses the relationship between subpopulations relative to their allele frequencies, inbreeding, or rate of exchange of individuals, were sufficiently low (<0.05) to suggest at least occasional migration or reciprocal gene flow among populations. Panmixia cannot be confirmed, because rates of gene flow as low as a few fish per generation are sufficient to prevent population differentiation by genetic drift (Wright, 1931; Hauser and Ward, 1998).

Based on the genetic marker tested, the null hypothesis that kingfish within the ROPME sea area constitute a single panmictic stock cannot be rejected. This assumption is further supported by microsatellite studies (LvH, unpublished data), which revealed little genetic substructure (F_{ST}: range 0.010−0.055) in kingfish ($n = 298$) sampled from six areas along the coasts of Oman and Yemen, and at the entrance of the Gulf (Bandar Abbas, Iran), evidence consistent with high gene flow in an admixed single stock. Some of these areas correspond closely to the DIB, OMN, and RAK sampling locations in the present study, although the sample locations used in the unpublished data set mentioned above did not allow comparison of populations inside and outside the Gulf.

The general absence of noticeable barriers to dispersal in the marine environment effectively reduces heterogeneity among populations, often making it difficult to differentiate discreet populations. This is particularly true of highly migratory vagile species with planktonic larvae (e.g. kingfish), and is reflected in the observed low F_{ST} values of the present study. Typically, F_{ST} values for marine fish are low, with a reported median of only 0.020 (Waples, 1998). Values <0.050 suggest restricted gene flow among populations, permitting some population subdivision. Clearly, the F_{ST} values for the kingfish RFLP and sequence pairwise comparisons are very low and, based on the marker tested, are consistent with negligible population genetic substructure in the ROPME sea area (Table 3). Significant values of p for F_{ST} between DIB and KUW indicated that there are unique characters in the DIB population. However, this is not reflected in the OMN samples, which are also outside the Gulf. A noticeable reduction in the AUH:DIB F_{ST} value (Table 3) from 0.052 (RFLP data) to 0.013 (sequence data) most likely resulted from a sampling artefact caused by the loss of an *Nla*III restriction site for DIB when the fragment size was reduced to 308 usable nucleotide sites during sequence analysis.

The findings from the present study and similar investigations conducted elsewhere suggest that members of the genus *Scomberomorus* share characteristics of high vagility and admixture within their respective regions. For example, Buonaccorsi *et al.* (1999) reported homogeneous distribution of the sister species *S. maculatus* throughout the western Atlantic and Gulf of Mexico based on mitochondrial and nuclear DNA analyses. Moreover, Gold *et al.* (1997) reported similar homogeneity of another sister species, *S. cavalla*, from the same regions for mtDNA, but also identified weak divergence between the Atlantic and Gulf of Mexico using a nuclear-encoded dipeptidase locus (*PEPA-2*) allozyme analysis. In this case, the distribution of *PEPA-2* was consistent with a historical event caused by population subdivision during the Pleistocene glacial period. Broughton *et al.* (2002) further evaluated *S. cavalla* from the western Atlantic and Gulf of Mexico by comparing variation at five microsatellite loci, and concluded that the lack of heterogeneity between regions reflected reciprocal gene flow consistent with a single intermingling genetic stock.

There are inherent limitations to molecular markers, and it is important to consider their high sensitivity to low levels of gene flow (Carvalho and Hauser, 1994). As just a few intermingling fish per generation are capable of preventing population differentiation, it is possible to misinterpret the genetic stock structure and to infer panmixia, even though it may not be the case. Caution is also advised against making untested genetic substructure comparisons of similar species sharing the same habitat. In particular, it should not be assumed that species sharing taxonomic or ecological characteristics will exhibit similar spatial genetic substructure (Graves, 1998). For example, genetic analysis of three closely related highly migratory species

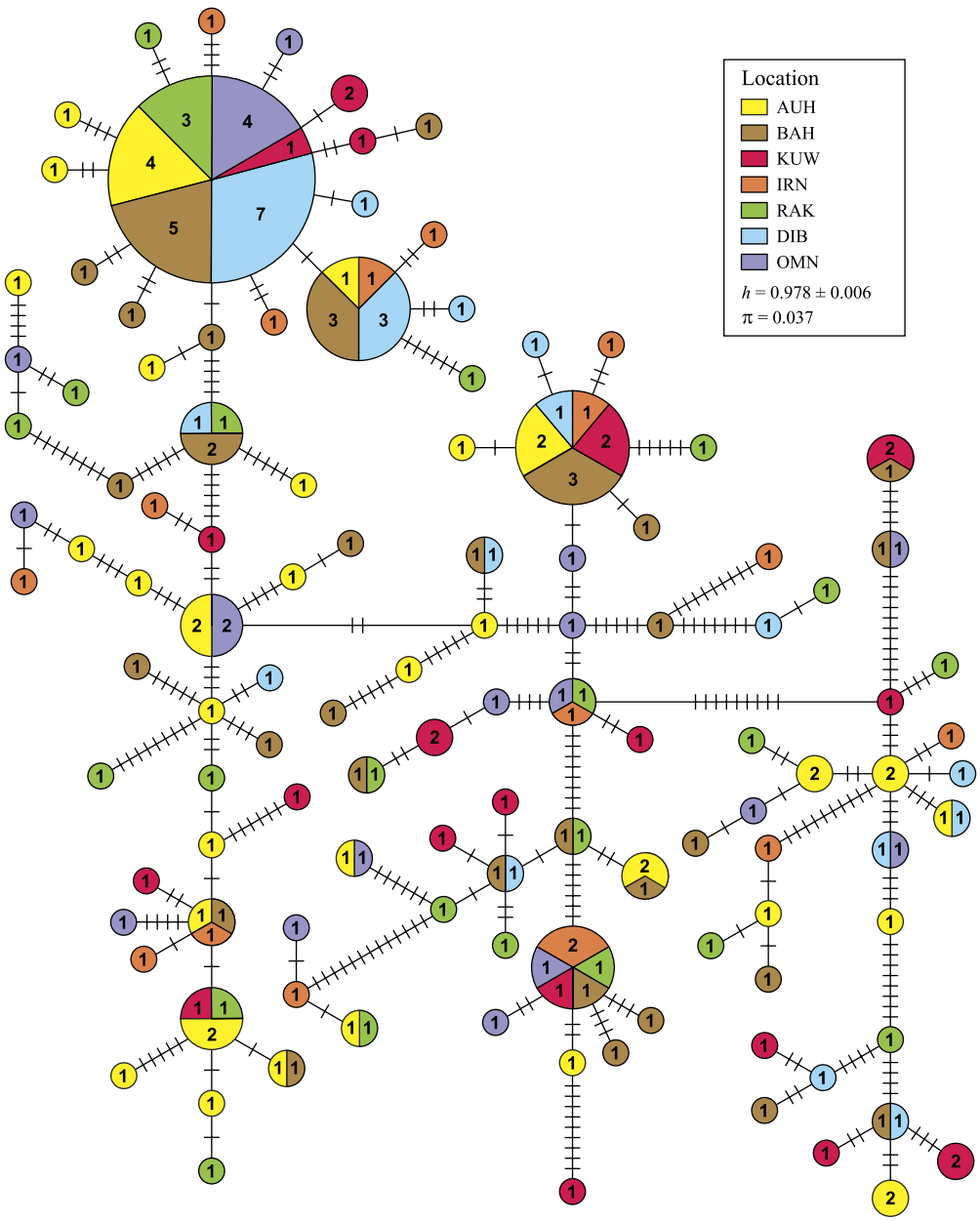

Figure 3. Bubble diagram of minimum spanning tree showing relationships of 116 mtDNA sequence haplotypes derived from 193 *Scomberomorus commerson* individuals from seven geographic locations in the ROPME sea area. Colours denote sampling locations. Circles indicate haplotypes, while circle size and numbers indicate the number of individuals sharing a particular haplotype. Crossbars on connecting lines denote number of substitutions separating haplotypes. Overall haplotype (h) and nucleotide (π) diversities are shown.

of tuna (yellowfin, *Thunnus albacares*; bigeye, *T. obesus*; and albacore, *T. alalunga*), with similar global distributions, exhibited very different genetic stock structures (Ward, 2000). Furthermore, in the ROPME sea area, the evidence of a genetically isolated population of sailfish living year-round inside the Gulf illustrates that not all highly migratory species sharing the same habitat will similarly share characteristics of movement behaviour or genetic stock structure (Hoolihan *et al.*, 2004). Indeed, based on the genetic marker analysed, these results suggest that the Strait of Hormuz is not an effective impediment to the movement of kingfish in and out of the Gulf. However, evidence of spatial substructure might be revealed by testing larger samples and additional neutral markers.

Although detailed understanding of movement and spawning patterns of kingfish is lacking, the anecdotal

claims that they migrate in and out of the Gulf (Al-Oufi *et al.*, 2004), along with the apparent lack of spatial boundaries suggested by genetic analyses, support a single-stock management regime for kingfish within the ROPME sea area, at least until further studies demonstrate otherwise. One important consideration, as described by Avise (1994), is that genetic marker distribution may retain evolutionary and demographic characteristics different from contemporary behavioural characteristics. In other words, the tested population may be moving and dispersing in patterns other than that indicated by the neutral genetic marker. An important point to consider is that stock structure may be present if adults exhibit natal homing, but can remain undetected if captures occur during periods of more extensive mixing. Sex was not differentiated in the present study, so it is unknown whether the movements and dispersal patterns of males and females are identical. Ovenden and Street (in press) report that *S. commerson* in Australia exhibit sex-biased dispersal, with females being more philopatric and males more wide-ranging. Therefore, a thorough understanding of behavioural characteristics is needed to corroborate genetic evidence. Conventional and electronic tagging, parasite analysis, and otolith chemistry are possible alternative methods for determining behavioural movement and dispersal. In the case of Gulf sailfish, the genetic evidence for isolation was supported by spatial distribution of tag recaptures in mark-recapture studies (Hoolihan, 2003; Hoolihan *et al.*, 2004).

Recent reports suggesting that kingfish may be overexploited in the ROPME sea area (Al-Oufi *et al.*, 2004; Grandcourt *et al.*, 2005) stress the importance of establishing a regional cooperative approach to shared management that best serves the interests of all nations and stakeholders. More rigorous studies on life history, tagging, and advanced genetics are recommended to gain better understanding of the biology and essential requirements of this regionally important species.

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