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Intraspecific phylogeographic isolation of Arabian Gulf sailfish *Istiophorus platypterus* inferred from mitochondrial DNA

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Abstract The genetic structure of seven sailfish *Istiophorus platypterus* populations sampled from three locations inside and four locations outside the Arabian Gulf was determined by restriction fragment length polymorphism analysis of mitochondrial DNA of 147 individuals using eight restriction endonucleases. A total of 39 composite haplotypes derived from 27 presumptive restriction sites demonstrated significant differences in frequency between population groups inside and outside the Gulf (analysis of molecular variance 34.80%, $P < 0.001$; $F_{ST} = 0.356$) and evidence of restricted migration between them (average number of migrants, $N_m = 0.903$). Haplotypes found only inside or outside the Gulf clustered to all major branches of a haplotype phylogeny, as did those found in both areas. The reduced genetic diversity of the Gulf populations and the fact that much of the differentiation between the population groups resulted from differences in haplotype frequency rather than divergence between haplotypes suggest a founder effect and a recent sampling of genotypes from the Indian Ocean. This was probably associated with dispersal into the Gulf after it was flooded by rising sea level after the end of the last glaciation around 8,000 years ago. At some point since then the population has evolved to complete its life cycle within the Gulf and shows a marked disruption to gene flow, consistent with dispersal data, at the Strait of Hormuz. These

findings represent the first clear evidence of phylogeographic isolation occurring in a large, highly vagile, predatory istiophorid billfish, within a marginal sea.

Introduction

Recent findings suggest that global stocks of large pelagic fish such as tunas and billfishes are in alarming decline due to overexploitation (Myers and Worm 2003). An understanding of population genetic variation is important for successful conservation management, and genetic analyses have proven effective for determining fish population structure at intraspecific levels (Féral 2002; Palumbi 1994; Ward 2002) and identifying component evolutionary lineages in fish populations (Birmingham and Moritz 1998; Moritz and Faith 1998). Investigations of genetic stock structure in billfish (families Istiophoridae, Xiphiidae) are not abundant, but the benefits of using genetic tools have been demonstrated for various purposes including species identification, management, population structure, evolution, and forensic evaluation (Buonaccorsi et al. 1999; Finnerty and Block 1992, 1995; Graves 1998; Graves and McDowell 1994, 1995, 1998, 2003; Innes et al. 1998; McDowell and Graves 2001, 2002).

Spatial structure in billfish populations has been observed but usually at large spatial scales. Analyses of Atlantic Ocean sailfish mitochondrial DNA (mtDNA) and microsatellites did not reveal significant stock structure, suggesting samples within that ocean share a single gene pool (Graves and McDowell 1998; McDowell and Graves 2001). Significant spatial partitioning of genetic variation has been described in geographically distant striped marlin *Tetrapterus audax* within the Pacific Ocean using restriction fragment length polymorphism (RFLP) analysis of mtDNA (Graves and McDowell 1994), between populations of blue marlin and white marlin *T. albidus* in different oceans (Graves and McDowell 1995), between stocks of the swordfish

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Xiphias gladius (Chow and Takeyama 2000; Chow et al. 1997), and in Pacific and Indian Ocean sailfish *Istiophorus platypterus* populations, both between and within these oceans (unpublished data, cited in Graves and McDowell 2003).

Sailfish are found worldwide in tropical and subtropical waters (Nakamura 1985) and are the only billfish species inhabiting the Arabian Gulf (also called the Persian Gulf; hereafter referred to simply as the Gulf). In the United Arab Emirates (UAE), the sailfish is an important recreational gamefish, as in many other areas of the world. In adjoining Iranian territorial waters sailfish are taken commercially, primarily as bycatch in the gillnet fishery targeting other species (Hoolihan 2004). A cooperative tagging program initiated in 1998 (Hoolihan 2001) has had a recovery rate exceeding 6% (98 of 1,615) and all recaptures have come from inside the Gulf, even though fishing activities and awareness of the tagging program existed outside the Gulf (Hoolihan 2003). Assuming the tag recapture data is an accurate representation of movement patterns the data suggest Gulf sailfish may be resident in the Gulf and isolated from populations in the Indian Ocean. This would be a unique circumstance given the large spatial scales at which variation has been detected between sailfish populations to date (Graves and McDowell 2003).

The Gulf is a shallow semi-enclosed basin of 337 km maximum width and approximately 1,000 km in overall length with a single restricted seawater inflow/outflow at the Strait of Hormuz (56 km wide) connecting to the Gulf of Oman, and further to the Arabian Sea and Indian Ocean (Reynolds 1993). The restricted access through the Straits of Hormuz may represent a barrier to the movement of marine biota, but this would also be unusual for a large pelagic fish.

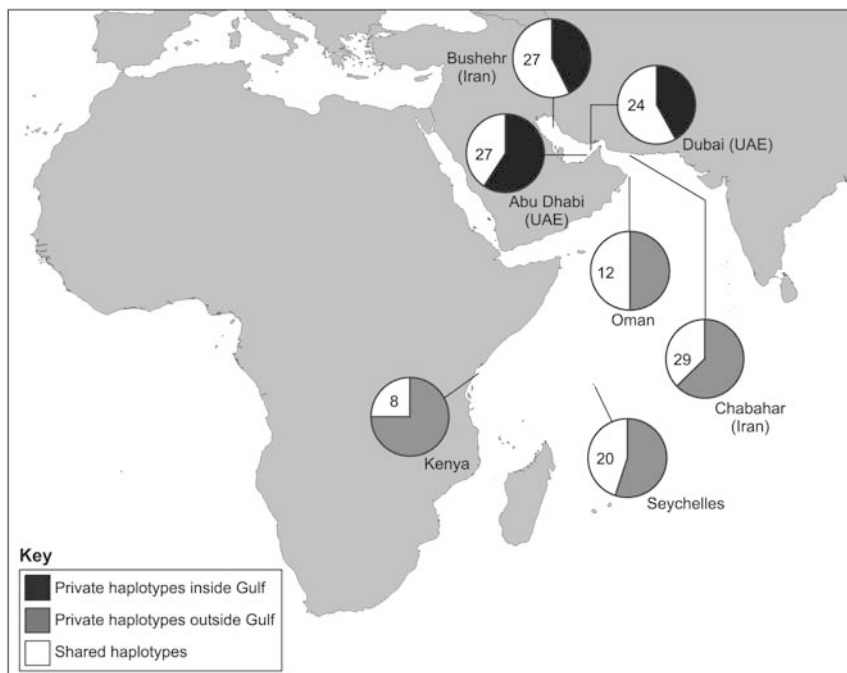
This article reports the analysis of genetic variation at the hypervariable control region (D-loop) of sailfish mtDNA to test the null hypothesis that sailfish inside and outside the Gulf constitute a single panmictic stock, and to test whether there is any evidence supporting spatial partitioning of genetic variation.

Materials and methods

Tissue samples were excised from the antero-dorsal edge of dorsal fin I from recreational and commercial sailfish landings, as well as tagged and released specimens. Samples were preserved in a DMSO-based storage buffer medium (Seutin et al. 1991). A total of 147 sailfish were sampled from three geographical locations inside the Gulf and four outside (Fig. 1). Locations inside included the UAE (Abu Dhabi 24°48'N, 54°12'E, Dubai 25°20'N, 54°46'E) and Iran (Bushehr 28°56'N, 50°49'E), while those outside included Iran (Chabahar 25°24'N, 60°48'E), Oman (Sur 22°30'N, 59°30'E), Kenya (Malindi 03°12'S, 40°06'E), and Seychelles (Mahe 04°42'S, 55°30'E).

DNA extraction was facilitated by DNeasy tissue kits (Qiagen) as per the manufacturer's instructions, with one modification: the volume of proteinase K solution was increased from 20 to 40 μ l (20 mg/ml). Tissue samples (25 mg) were digested overnight in a 55°C water bath. The polymerase chain reaction (PCR) was performed using Ready-to-go PCR beads (Amersham Pharmacia Biotech, Uppsala, Sweden) containing 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each of each dNTP, and 1.5 units of Taq DNA polymerase in a 25.0- μ l reaction. A single fragment of approximately 1,800 base pairs including the D-loop was amplified by using 200 ng

Fig. 1 Locations of seven sailfish populations sampled. The pie diagrams illustrate haplotype frequencies that are shared or private (inside or outside the Gulf only) found in 39 composite haplotypes from 147 sailfish. Private haplotypes account for 51% and 59% of samples inside and outside, respectively. Numbers in diagrams indicate sample size. See Table 3 for specific haplotype distribution and frequencies



of DNA and 10 pmol/ μ l of the universal primers CB3RL (5'CATATTAACCCGAATGATATTT3') and 12S ARH (5'ATAGTGGGGTATCTAATCCCAGTT3') as described by Palumbi et al. (1991) using a Progene (Techne Cambridge Ltd., UK) thermal cycling machine. Initial denaturation for 5 min at 95°C was followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 1 min 10 s, extension at 72°C for 2 min, and a final extension of 72°C for 10 min. PCR product was verified by electrophoresis of a 5- μ l volume in a 1% agarose/1% Synergel minigel for 30 min at 150 V (300 mA, 50 W) in 1X TAE buffer, stained with ethidium bromide, and visualized by UV transilluminator.

A minimum of 21 individuals were screened using 25 restriction endonucleases, targeting the D-loop region. The preliminary analysis revealed eight polymorphic endonucleases (*Sau3A I*, *Hinf I*, *Sty I*, *Bsp 1286 I*, *Afa I*, *Mva I*, *Fok I*, and *Hph I*) and these were used to screen all 147 samples. Restriction digestion was carried out in a 4.0- μ l volume containing 3.2 μ l of PCR product, 0.4 μ l restriction enzyme (four units) and 0.4 μ l of the appropriate buffer. All enzymes were products of Amersham Pharmacia Biotech (Uppsala, Sweden) except *Fok I* and *Hph I*, which came from New England Biolabs, Inc. (Beverly, Mass., USA). RFLP digestion was performed in a water bath at 37°C for 75 min. Restriction fragments were separated on GeneGel Excel 12.5% polyacrylamide gels (Pharmacia Biotech, Uppsala, Sweden) under conditions of 15°C, 600 V, 25 mA, 15 W, and visualized by silver staining (Pharmacia Biotech, Uppsala, Sweden).

Data analysis

Population analysis

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) programs available in Arlequin (Schneider et al. 2000) were used to calculate F_{ST} and hierarchical analysis of Φ_{ST} (analogous to F_{ST}) and calculate mismatch distributions (Rogers and Harpending 1992) for individual populations, as well as pooled populations inside and outside the Gulf. The D and DA modules in REAP (McElroy et al. 1992) were used to estimate nucleotide diversity (π) within populations and nucleotide divergence (d_{xy}) among populations (Nei and Tajima 1981). Tajima's D statistic (Tajima 1989a) for testing selective neutrality was calculated to assess evidence of population expansion (Tajima 1989b). Chakraborty's test of population amalgamation (Chakraborty 1990) was used to calculate evidence of population mixing. An exact test of population differentiation was calculated, as described by Raymond and Rousset (1995) and Goudet et al. (1996). The Mantel test statistic Z (Mantel 1967) was calculated in the program TFPGA (Miller 1997) to assess the correlation between F_{ST} and geographical distance between populations. The geographical distance between each population pair was estimated by calculat-

ing the shortest reasonable straight-line swimming route linking locations.

Haplotype analysis

A character state matrix showing the presence or absence of presumptive restriction sites created in Arlequin (Schneider et al. 2000) was used to construct a minimum spanning treefile between the 39 operational taxonomic units, or composite haplotypes, illustrated (see Fig. 4) with the program Treeview (Page 1996).

Results

A total of 27 presumptive restriction sites were observed (Table 1), giving a total of 39 composite haplotypes (Table 2) of which 14 were found inside the Gulf ($n = 78$) and 33 outside the Gulf ($n = 69$). At the population level, 24 (61.5%) are private haplotypes (i.e. occurred in only one population). Just 5 haplotypes (h12, h26, h32, h34, h37) account for 64 (82%) individuals from inside the Gulf, and 28 (35.8%) of these represent a single haplotype (h26) not observed outside the Gulf (Table 3). The populations inside the Gulf also contain 9 (11.5%) individuals possessing a unique private restriction site (c) for *Sau3A I* (Table 1). Populations outside the Gulf have a private haplotype (h3) found in 12 (17.4%) sailfish. Haplotype h4 is found in both groups but at very different frequencies (inside 5%, outside 18.8%). The remainder of the populations outside the Gulf is composed of haplotypes occurring in 1 to 5 individuals. The numbers of different private haplotypes found inside and outside are 6 and 25, respectively. In total, 51% of the haplotypes found inside the Gulf and 59% of those found outside the Gulf are private to their respective areas (Fig. 1).

Genetic diversity within populations

Nucleotide diversity (π) estimates for the seven populations ranged from 0.022 (Oman) to 0.051 (Chabahar) with populations pooled into two groups (inside and outside the Gulf) having nearly equivalent values of 0.039 and 0.041, respectively (Table 4). Chabahar and Oman had higher numbers of haplotypes than the other populations, but the ratio of the number of haplotypes (n_h) to the number of individuals sampled (n_i), n_h/n_i , and haplotype diversity (h), were generally lower for populations within the Gulf than those outside, and this is reflected clearly in the different values for the pooled populations inside and outside the Gulf (Table 4).

Genetic differentiation between populations

The marked differences in frequency of several haplotypes inside and outside the Gulf, detailed in the first

Table 1 Composite mitochondrial DNA (mtDNA) haplotypes observed among 147 sailfish. The 39 haplotypes represented 27 putative cutting sites

Composite haplotype no.	<i>Sau3A</i> I abc	<i>Hinf</i> I def	<i>Sty</i> I g	<i>Bsp1286</i> I hijkl	<i>Afa</i> I mnop	<i>Mva</i> I qr	<i>Fok</i> I stuvw	<i>Hph</i> I xyzA
h1	110	110	1	10000	1101	10	10000	1010
h2	110	110	1	10000	1101	10	10000	1001
h3	110	110	1	10000	1101	10	01000	1001
h4	110	110	1	10000	1101	10	01011	1001
h5	110	110	1	10000	1101	10	01011	0010
h6	110	110	1	10000	1101	10	00011	1010
h7	110	110	1	10000	1101	10	00011	1001
h8	110	110	1	10000	1111	10	01011	1001
h9	110	110	1	10000	1101	10	01000	1001
h10	110	110	1	11000	1101	10	01000	1001
h11	110	110	1	11000	1101	10	01011	0010
h12	110	110	1	11000	1101	11	10000	1010
h13	110	110	1	11000	1101	11	00011	1010
h14	110	110	1	10010	1100	10	01011	1001
h15	110	110	1	10001	1101	10	00011	1001
h16	110	110	0	10000	1101	10	00011	1010
h17	110	110	0	10000	1101	11	00011	1010
h18	110	110	0	11000	1101	10	00011	1001
h19	110	110	0	11000	1101	11	10000	1001
h20	110	110	0	11000	1101	10	00011	1010
h21	110	110	0	11000	1100	11	00011	1001
h22	110	100	1	10000	1101	10	10000	1001
h23	110	100	1	10000	1101	10	01011	1001
h24	110	100	1	10000	1101	10	00011	1001
h25	110	100	1	11000	1101	10	01000	1001
h26	110	100	1	11000	1101	11	10000	1010
h27	100	110	1	10000	1101	10	01000	1001
h28	100	110	1	10000	1101	10	00001	1001
h29	100	110	1	10100	1101	10	01011	1100
h30	100	110	0	11000	1101	10	10000	1010
h31	100	110	0	11000	1101	11	10000	1010
h32	100	110	0	11000	1101	11	00011	1010
h33	100	100	1	10000	1101	10	00001	1001
h34	100	100	0	11000	1101	11	00011	1010
h35	100	111	1	10000	1101	10	00111	1001
h36	101	110	1	11000	1101	11	10000	1010
h37	101	100	1	11000	1101	10	10000	1010
h38	001	110	1	10000	1101	10	01000	1001
h39	001	110	1	10000	1100	10	00011	1001

section of the Results, are clearly reflected in the pairwise F_{ST} values. All comparisons of populations within the Gulf, and all comparisons of populations outside the Gulf, with the exception of the Kenya–Oman comparison, were not statistically significant, while all comparisons of populations between these two groups were highly significant except the comparison of Chabhar and Dubai (Table 5). This pattern was repeated in the exact test of population differentiation (non-differentiation exact P values) where none of the within-group comparisons were statistically significant and all those comparing populations between groups were highly significant (data not shown). An AMOVA hierarchical analysis showed that most of the variation in haplotype frequencies could be attributed to variance within groups (62.49%, $P < 0.01$), followed by variation among groups (34.80%, $P < 0.001$). Only 2.72% ($P < 0.01$) was attributed to variation among populations. No significant relationship between the value of F_{ST} and geographical separation was observed among populations in the total data set (Mantel test: $Z = 11921.9$, $P = 0.93$), among populations inside the

Gulf ($Z = 44.6$, $P = 1.000$), or outside the Gulf ($Z = 786.2$, $P = 0.64$; Fig. 2).

Population history

The mismatch frequency distributions observed for the individual populations largely reflected the pattern for the pooled group to which they were associated (Fig. 3). The pooled population inside the Gulf produced a mismatch distribution with a bimodal pattern. None of the mismatch frequency distributions showed a smooth unimodal curve indicating sudden population expansion (Rogers and Harpending 1992) with the possible exception of Oman. No individual or pooled population had a significant negative value for Tajima's D , consistent with a sudden population expansion (values for individual populations ranged from -1.184 to 1.690 ; value for the pooled Gulf population was 1.711 , and for the pooled populations outside the Gulf -0.165). Only the pooled populations outside the Gulf had significantly more alleles (33) than expected (23) using Cha-

Table 2 Distribution of 39 composite mtDNA haplotypes. *Letters* reflect endonucleases *Sau3A I*, *Hinf I*, *Sty I*, *Bsp 1286 I*, *Afa I*, *Mva I*, *Fok I* and *Hph I* (left to right)

No.	Composite haplotype	Inside Gulf				Outside Gulf					Total all
		Abu Dhabi	Dubai	Bushehr	Total inside	Chabahar	Oman	Seychelles	Kenya	Total outside	
h1	AAAAAAA	0	0	1	1	0	0	0	0	0	1
h2	AAAAAAB	0	0	0	0	1	0	0	0	1	1
h3	AAAAAAB	0	0	0	0	4	2	6	0	12	12
h4	AAAAACB	0	3	1	4	6	2	4	1	13	17
h5	AAAAACD	0	1	0	1	1	1	0	0	2	3
h6	AAAAADA	0	1	0	1	0	0	0	0	0	1
h7	AAAAADB	1	2	0	3	0	2	2	1	5	8
h8	AAAABACB	0	0	0	0	0	1	0	0	1	1
h9	AAAACABB	0	0	0	0	1	0	0	0	1	1
h10	AAABAABB	0	0	0	0	0	0	0	3	3	3
h11	AAABAACD	1	0	0	1	0	0	0	0	0	1
h12	AAABABAA	4	2	4	10	1	0	0	0	1	11
h13	AAABABDA	0	0	0	0	1	0	0	0	1	1
h14	AAADCACB	0	0	0	0	0	0	1	0	1	1
h15	AAAEAADB	0	0	0	0	0	2	0	0	2	2
h16	AABAAADB	0	0	0	0	1	0	0	0	1	1
h17	AABAABDA	0	0	0	0	1	0	0	0	1	1
h18	AABBAADB	0	0	0	0	0	1	0	0	1	1
h19	AABBABAB	0	0	0	0	0	0	1	0	1	1
h20	AABBABDA	0	0	0	0	1	0	0	0	1	1
h21	AABBCBDB	0	0	0	0	0	0	0	1	1	1
h22	ABAAAAAB	0	0	0	0	1	0	0	0	1	1
h23	ABAAAACB	0	1	0	1	0	1	1	0	2	3
h24	ABAAAADB	1	0	0	1	1	0	0	0	1	2
h25	ABABAABB	0	0	0	0	0	0	1	0	1	1
h26	ABABABAA	13	7	8	28	0	0	0	0	0	28
h27	BAAAAAAB	0	0	0	0	0	0	0	2	2	2
h28	BAAAAAEB	0	0	0	0	1	0	0	0	1	1
h29	BAACAACC	0	0	0	0	1	0	0	0	1	1
h30	BABBAAAA	0	0	0	0	1	0	0	0	1	1
h31	BABBABAA	0	0	0	0	2	0	0	0	2	2
h32	BABBABDA	2	5	5	12	2	0	1	0	3	15
h33	BBAAAAEB	0	0	0	0	1	0	0	0	1	1
h34	BBBBABDA	3	0	3	6	0	0	1	0	1	7
h35	BCAAAAFB	0	0	0	0	0	0	1	0	1	1
h36	CAABABAA	0	0	1	1	0	0	0	0	0	1
h37	CBABAAAA	2	2	4	8	0	0	0	0	0	8
h38	DAAAAAAB	0	0	0	0	0	0	1	0	1	1
h39	DAAACADB	0	0	0	0	1	0	0	0	1	1
Total		27	24	27	78	29	12	20	8	69	147

kraborty's test, indicating mixing of populations in that case. The difference in the number of observed and expected alleles was less than three in all other comparisons and not statistically significant.

Haplotype phylogeny

The parsimony analysis of the 50% consensus tree showed two major clusters, the smaller of which was dominated by haplotypes from inside the Gulf (Fig. 4). It was composed of seven haplotypes (h1, h12, h26, h30, h31, h36, and h37) that accounted for 52 (66%) samples from inside the Gulf, and 4 (5.8%) from outside the Gulf. However, haplotypes found either solely inside or outside the Gulf or those that were found in both areas are distributed over all major branches of the tree. When this tree was illustrated as a bubble diagram, the lack of

any deep evolution among haplotypes is re-emphasized (Fig. 5).

Discussion

The analysis has demonstrated significant genetic differences between populations inside and outside the Gulf ($F_{ST}=0.356$) providing an estimate of the average number of migrants per generation between these areas of less than one ($N_m=0.903$). The null hypothesis that the Gulf and Indian Ocean areas constitute a single panmictic stock can be rejected. The genetic findings are consistent with published tag recapture data (Hoolihan 2003), and both are consistent with a break at the Strait of Hormuz.

The distribution of haplotypes found only from within the Gulf, only outside the Gulf, and in both areas

Table 3 Relative frequencies of composite haplotypes

Haplotype	Inside Gulf				Outside Gulf				
	Abu Dhabi <i>n</i> = 27	Dubai <i>n</i> = 24	Bushehr <i>n</i> = 27	Frequency inside	Chabahar <i>n</i> = 29	Oman <i>n</i> = 12	Seychelles <i>n</i> = 20	Kenya <i>n</i> = 8	Frequency outside
h1	0	0	0.037	0.013	0	0	0	0	0
h2	0	0	0	0	0.035	0	0	0	0.014
h3	0	0	0	0	0.138	0.167	0.300	0	0.174
h4	0	0.125	0.037	0.051	0.207	0.167	0.200	0.125	0.188
h5	0	0.042	0	0.013	0.036	0.083	0	0	0.029
h6	0	0.042	0	0.013	0	0	0	0	0
h7	0.037	0.083	0	0.038	0	0.167	0.100	0.125	0.072
h8	0	0	0	0	0	0.083	0	0	0.014
h9	0	0	0	0	0.035	0	0	0	0.014
h10	0	0	0	0	0	0	0	0.375	0.043
h11	0.037	0	0	0.013	0	0	0	0	0
h12	0.148	0.083	0.148	0.128	0.035	0	0	0	0.014
h13	0	0	0	0	0.035	0	0	0	0.014
h14	0	0	0	0	0	0	0.050	0	0.014
h15	0	0	0	0	0	0.167	0	0	0.029
h16	0	0	0	0	0.035	0	0	0	0.014
h17	0	0	0	0	0.035	0	0	0	0.014
h18	0	0	0	0	0	0.083	0	0	0.014
h19	0	0	0	0	0	0	0.050	0	0.014
h20	0	0	0	0	0.035	0	0	0	0.014
h21	0	0	0	0	0	0	0	0.125	0.014
h22	0	0	0	0	0.035	0	0	0	0.014
h23	0	0.042	0	0.013	0	0.083	0.050	0	0.029
h24	0.037	0	0	0.013	0.035	0	0	0	0.014
h25	0	0	0	0	0	0	0.050	0	0.014
h26	0.481	0.292	0.296	0.359	0	0	0	0	0
h27	0	0	0	0	0	0	0	0.250	0.029
h28	0	0	0	0	0.035	0	0	0	0.014
h29	0	0	0	0	0.035	0	0	0	0.014
h30	0	0	0	0	0.035	0	0	0	0.014
h31	0	0	0	0	0.069	0	0	0	0.029
h32	0.074	0.208	0.185	0.154	0.069	0	0.050	0	0.043
h33	0	0	0	0	0.035	0	0	0	0.014
h34	0.111	0	0.111	0.077	0	0	0.050	0	0.014
h35	0	0	0	0	0	0	0.050	0	0.014
h36	0	0	0.037	0.013	0	0	0	0	0
h37	0.074	0.083	0.148	0.103	0	0	0	0	0
h38	0	0	0	0	0	0	0.050	0	0.014
h39	0	0	0	0	0.035	0	0	0	0.014
Total	0.999	1	0.999	1.001	1.009	1	1	1	0.987

Table 4 Measures of genetic diversity within populations: 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 of sailfish sampled from locations inside and outside the Gulf (see Fig. 1)

Population	n_i	n_h	n_h/n_i	$h \pm SE$	π
Abu Dhabi	27	8	0.296	0.746 ± 0.078	0.032
Dubai	24	9	0.375	0.866 ± 0.044	0.048
Bushehr	27	8	0.296	0.849 ± 0.038	0.035
Chabahar	29	19	0.655	0.943 ± 0.029	0.051
Oman	12	8	0.667	0.939 ± 0.048	0.022
Seychelles	20	11	0.550	0.884 ± 0.054	0.038
Kenya	8	5	0.625	0.857 ± 0.108	0.029
Inside Gulf	78	14	0.179	0.820 ± 0.031	0.039
Outside Gulf	69	33	0.478	0.930 ± 0.019	0.041

across all branches of the consensus parsimony tree indicates that sailfish inside the Gulf represent a recent sub-sample of the sailfish outside. The lower number of haplotypes, and the lower ratio of the number of haplotypes to the number of sampled individuals (n_h/n_i) inside the Gulf (0.179) compared with outside (0.478) is

compatible with a recent sub-sample entering the Gulf having the genetically atypical structure expected of a small founding population. The mismatch distribution for the pooled population inside the Gulf was a bimodal curve showing an initial steep decline expected of a sudden population reduction (Rogers and Harpending

Table 5 Population pairwise F_{ST} s for seven populations below diagonal, geographical separation between populations in kilometers above diagonal

	Abu Dhabi	Dubai	Bushehr	Chabahar	Oman	Seychelles	Kenya
Abu Dhabi		82	568	808	897	3937	4437
Dubai	0.067 ^{n.s.}		559	726	815	3885	4355
Bushehr	-0.008 ^{n.s.}	0.053 ^{n.s.}		1285	1374	4414	4914
Chabahar	0.328 ^{***}	0.098 ^{n.s.}	0.302 ^{***}		347	3387	3887
Oman	0.582 ^{***}	0.323 ^{**}	0.560 ^{***}	0.082 ^{n.s.}		3040	3540
Seychelles	0.485 ^{***}	0.249 ^{**}	0.466 ^{***}	0.026 ^{n.s.}	0.023 ^{n.s.}		1720
Kenya	0.514 ^{***}	0.268 ^{**}	0.487 ^{***}	0.053 ^{n.s.}	0.144 ^{**}	-0.031 ^{n.s.}	

n.s., not significant; ** $P < 0.01$; *** $P < 0.001$

1992), followed by a distribution suggesting selection of a particular haplotype (Di Rienzo and Wilson 1991). Haplotype h26 accounts for 35.8% of the population inside the Gulf and is not found outside. If correct, this interpretation is compatible with a severe bottleneck and

founder effect associated with colonization of the Gulf and subsequent selection of particular haplotype(s) in that environment.

It is likely that historical climatic events were a factor in the dispersal and divergence of Gulf sailfish. Geological investigations indicate that during the most recent ice age (Pleistocene) regional sea water levels were 100–150 m lower; thus the Gulf was probably devoid of sea water (Reynolds 1993; Sheppard et al. 1992). An increase in global temperature commencing at the Holocene initiated glacial melting and Teller et al. (2000) reported that seawater flooded 1,000 km into the Gulf around the period of 14,000 to 8,000 years ago. Therefore, the probable period (~8,000–10,000 years) sailfish have inhabited the Gulf during the current flooding is comparatively short.

Phylogeographic breaks have been described as frequently coinciding with geographic boundaries (Avice 1994; Burton 1998). The most apparent characteristic of the Strait of Hormuz is its restricted physical size. However, this restriction affects other parameters in the Gulf that may constitute additional ecological barriers to gene flow. For example, low water circulation between the Gulf and outside, coupled with high temperature and evaporation rates, results in higher salinity levels inside the Gulf. Salinity entering the Gulf is approximately 36.5‰, whereas inside the Gulf average salinity is 38.0‰ and can reach as much as 43.0‰ in some areas (Brewer and Dyrssen 1985; Sultan and Elghribi 1996). Sea surface temperatures affected by winter winds have been recorded in the Gulf as low as 4°C, whereas extreme summer heat raises the temperature to around 34°C (Sheppard et al. 1992). These extremes are believed largely responsible for the much lower biodiversity levels found in the Gulf and could provide a template for the selection of particular haplotypes.

The shallow depth (mean 36 m) of the Gulf may also act as a deterrent to occupation by some species. For example, striped marlin, black marlin, blue marlin, and swordfish are present in all the locations sampled outside the Gulf, but only sailfish are found inside the Gulf. Marlins and swordfish are noted for their preference of deep waters and, on occasion when they are observed in shallow areas, it is invariably near a deep drop-off.

There is ample evidence of a marginal sea similar to the Gulf playing a role in the differentiation of other fish species. The Sea of Cortez (Gulf of California) is an isolated arm of the Pacific and has environmental characteristics contributing to disjunction of some

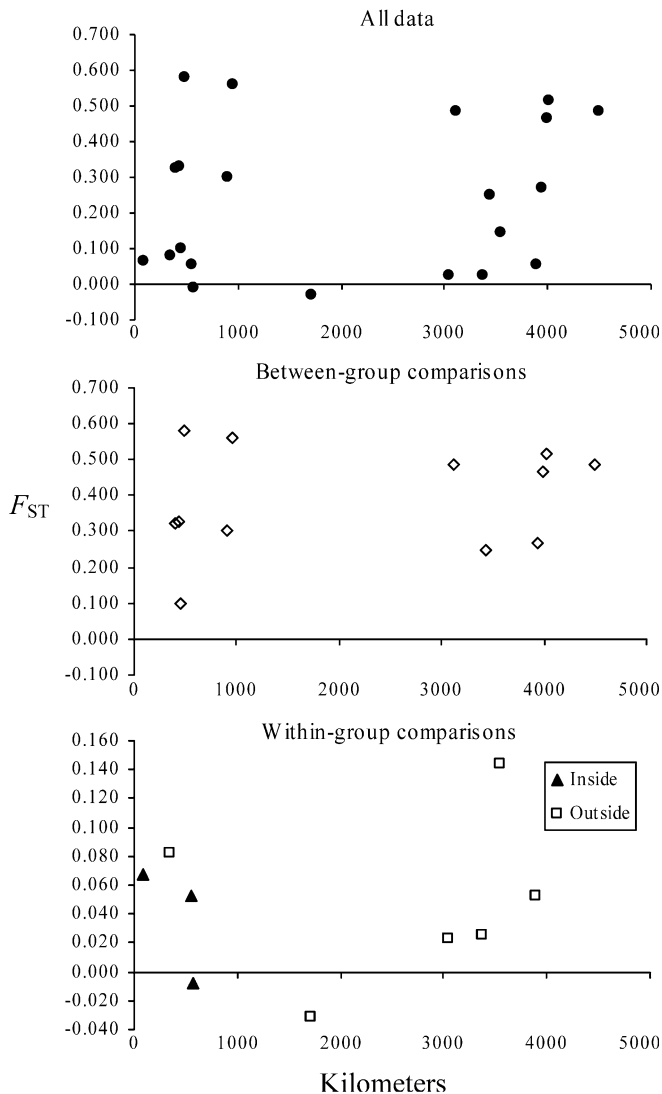
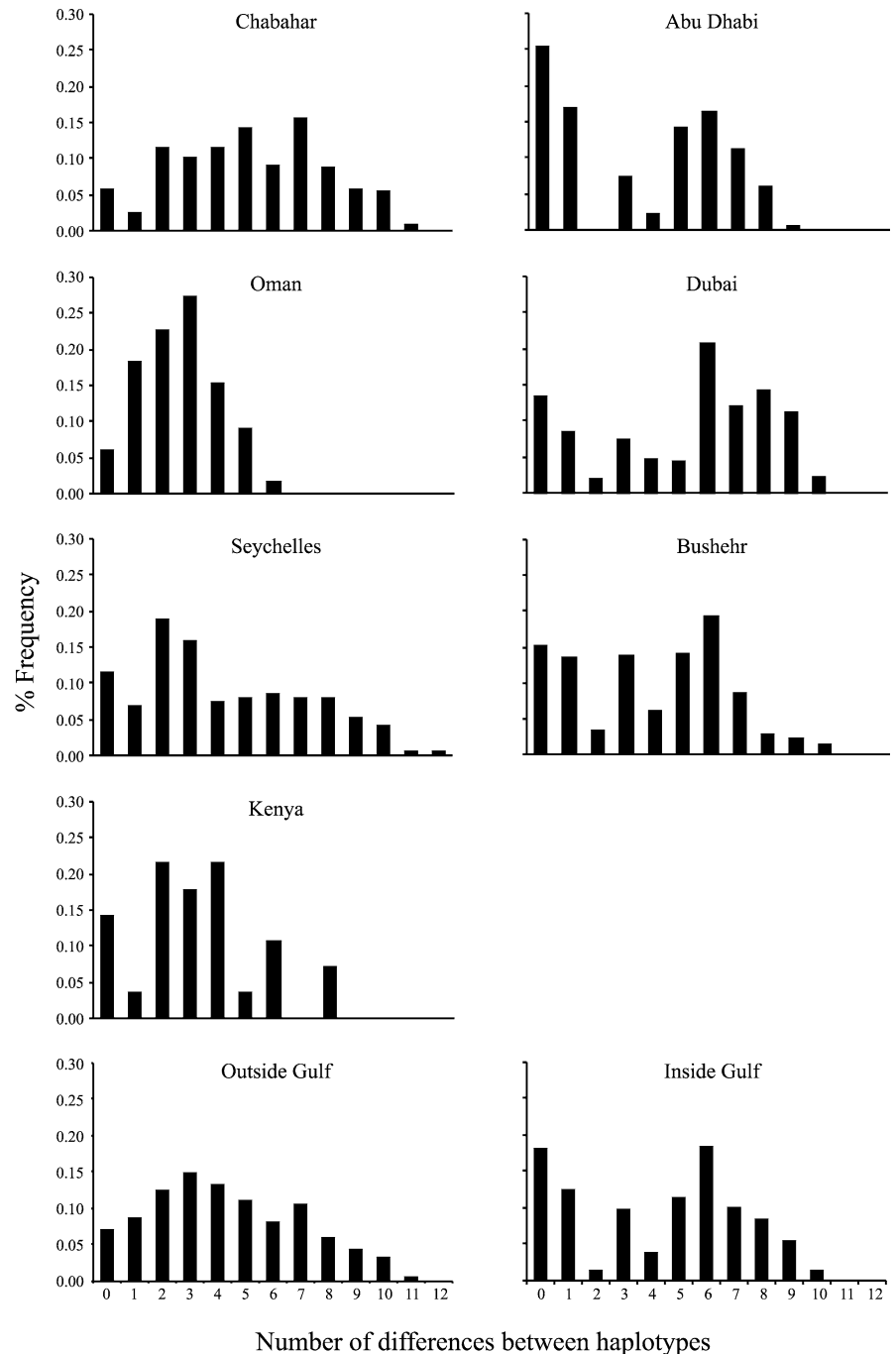


Fig. 2 Pairwise population differentiation (F_{ST}) plotted as a function of the geographical separation of the seven sailfish populations from inside and outside the Gulf. The top graph plots the data for all populations. The middle graph plots only comparisons of inside to outside. The bottom graph plots comparisons within inside and within outside

Fig. 3 Mismatch distributions for each of the seven sailfish populations sampled, plus pooled populations for inside and outside the Gulf



marine species. Fauna and conditions in the northern Sea of Cortez are more temperate, akin to properties found on the outer Pacific coast, as compared to the southern end near the mouth. The warmer conditions in the south form an ecological barrier to movement for some species. Cryptic speciation in the opaleye *Girella nigricans*, and genetic disjunction of reef fish (Terry et al. 2000) and sand bass *Paralabrax maculatofasciatus* (Stepien et al. (2001) have been reported between the Sea of Cortez and those populations along its outer Pacific coast boundary. Although the adults of these species are

far less vagile than billfishes, they do have pelagic larval stages aiding dispersal. It has been suggested that ecological barriers formed by temperature preference contribute to gene flow disruption in the Sea of Cortez (Stepien et al. 2001; Terry et al. 2000). Similarities between the Sea of Cortez and the Gulf include establishing ecological and geographical barriers from which preferential habitat conditions develop and influence regional phylogeographies, factors most likely contributing to Gulf sailfish isolation. Intra-ocean isolation by distance has been reported for blue marlin, striped

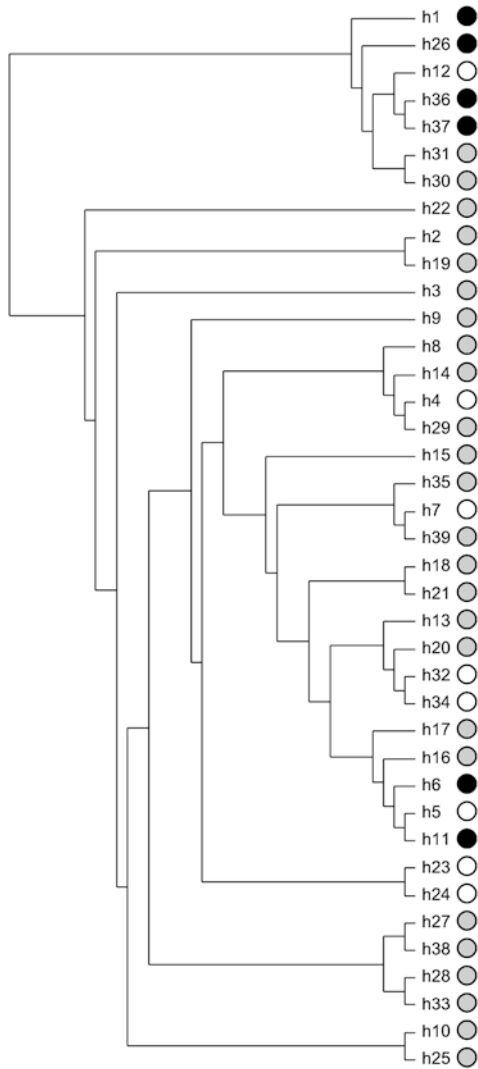


Fig. 4 Minimum spanning tree illustrating evolutionary relationships between haplotypes. *Black circles* indicate private haplotypes inside the Gulf, *shaded circles* private haplotypes outside, and *unshaded circles* shared haplotypes

marlin, and sailfish (Graves and McDowell 1994, 2003). However, no previous study has shown isolation of billfish within a marginal sea area, with the exception of swordfish in the Mediterranean (Chow and Takeyama 2000; Chow et al. 1997). The evidence presented here for Arabian Gulf sailfish represents the first report of intraspecific differentiation of an istiophorid billfish inhabiting a marginal sea area.

Commercial exploitation of Gulf sailfish has continued in the absence of compelling data that would indicate the population may be an isolated subdivision, therefore susceptible to depletion by over-fishing. The evidence of a clear disjunction of mtDNA haplotypes inside and outside the Gulf is consistent with isolation of the Gulf population and suggests the importance of treating the Gulf population as a distinct stock management unit of limited size, with particular consideration for protecting genetic diversity.

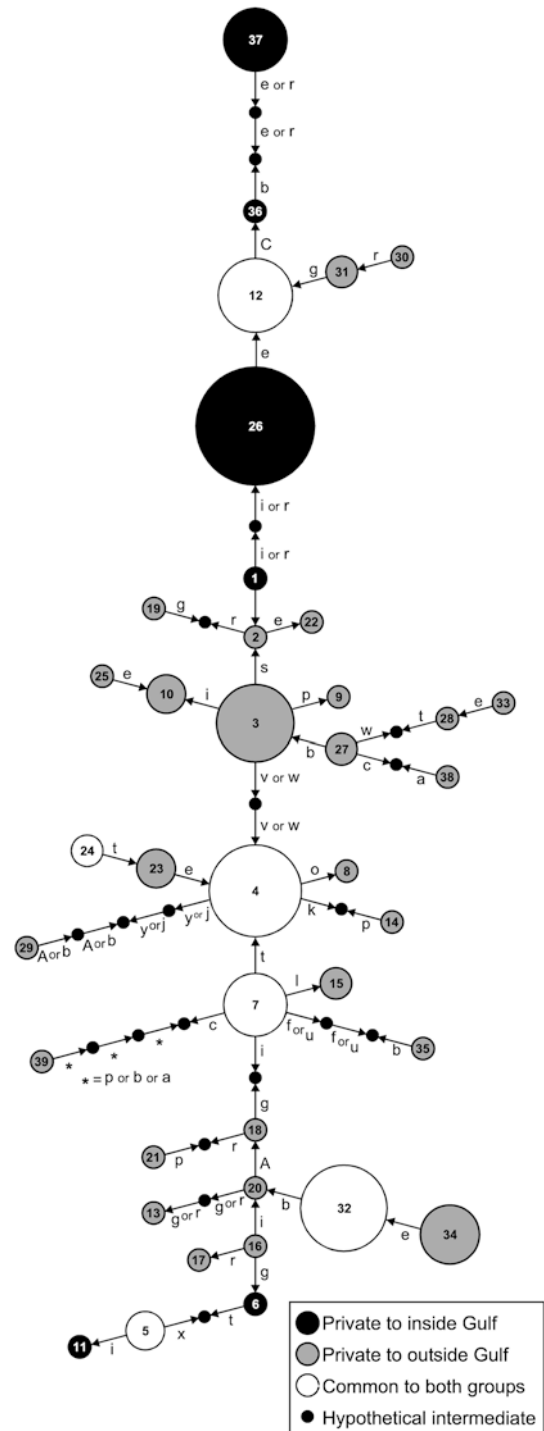


Fig. 5 Maximum likelihood network showing a possible evolutionary relationship among haplotypes of seven sailfish populations. *Large black circles* represent haplotypes private to inside the Gulf, *shaded circles* represent haplotypes private to outside the Gulf, and *unshaded circles* represent common haplotypes to both groups. *Small black circles* represent hypothetical haplotypes that are intermediate mutational steps inferred to link observed haplotypes where no direct intermediates were observed. The area of each circle represents the frequency with which the haplotype occurs. *Arrows* indicate the direction of loss of a restriction site. *Letters* indicate which site changed, and *numbers* refer to the composite haplotype (see Table 1)

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