

**Population Structure of Australian Swordfish,
*Xiphias gladius***

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Final Report to AFMA

April, 2001



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Preferred way to cite this publication:
Ward, R.D., Reeb, C.A. and Block, B.A. (2001) Population structure of Australian swordfish, *Xiphias gladius*. Final Report to the Australian Fisheries Management Authority, Canberra.

Published by CSIRO Marine Research.

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EXECUTIVE SUMMARY

The aims of this study were to examine the genetic stock structure of Australia's swordfish fisheries on the west and east coasts, and to determine how these populations might relate to other populations in the Indian and Pacific Oceans. It was decided to use a combination of mitochondrial DNA and microsatellite DNA analyses. Both these approaches had earlier been used to study swordfish stock structure by Dr Barbara Block and Dr Carol Reeb at Stanford University, California. A collaborative study was consequently established between CSIRO Marine Research and Stanford University.

Collections of swordfish were made from three areas: Australia's east coast fishery (off New South Wales and southern Queensland), Australia's west coast fishery (off Port Hedland and off Geraldton), and from off Reunion Island (west Indian Ocean, off Madagascar). Samples from the Pacific Ocean had already been collected and analysed by Stanford.

No statistically significant differences among the three project collections were detected for mitochondrial DNA. The null hypothesis that fish from Reunion Island, western Australia and eastern Australia form part of the same gene pool could not be rejected. However, it was interesting that two fish belonging to Clade II, a phylogenetic clade found in highest abundance in the Mediterranean Sea and Atlantic Ocean were in the western Australia collection. The remaining fish belonged to the more ubiquitous Clade I which is found distributed worldwide. The lack of Clade II in the Reunion Island collection likely reflects its small sample size. The presence of Clade II in the Indian Ocean might represent current or historical gene flow from the Atlantic Ocean.

Minor but significant differentiation in microsatellite allele frequencies over the three collections was recorded using one of three different analytic approaches. Much of the significant differentiation recorded by the AMOVA F_{ST} method was due to minor differentiation of the Western Australian population from the two other collections, at locus Xg-144.

Combing the mtDNA and nuclear DNA evidence with data previously published (Reeb et al. 2000) and unpublished genetic data (this study) from the Pacific suggests several stocks: (1) a northern Pacific stock (differentiated by mtDNA), (2) a southern Pacific stock (differentiated by mtDNA) which is, surprisingly, genetically similar to Reunion Island, and (3) a western Australia stock (differentiated by microsatellite DNA). However, if there are distinct stocks, then the degree of separation of them is small. A larger study of the genetics of swordfish of this area is required to finally resolve this issue.

2 INTRODUCTION

1.1. Background information on swordfish

Australian swordfish populations are being increasingly exploited. Most are taken in the eastern AFZ. Initially Japanese longliners took most of the catch, but in recent years their effort has decreased, and ceased in 1998. Catch in the eastern AFZ from domestic longliners was minimal in the early 1990's, but then increased sharply to more than 2000 t in each of 1998 and 1999 (see Table 1). Most of the domestic catch is taken from the Brisbane Grounds (an area bounded by 24-32°S and 152-162°E).

Table 1. Catch taken and catch per unit effort (CPUE, estimated as kg/1000 hooks) in the eastern AFZ over the last ten years (data from Ward and Elscot 2000)

Year	Domestic fishery		Japanese fishery	
	Catch (t)	CPUE	Catch (t)	CPUE
1990	21	19	1018	85
1991	56	38	853	146
1992	46	24	1078	181
1993	27	18	701	94
1994	36	15	645	85
1995	65	20	525	77
1996	613	165	291	72
1997	1881	332	405	62
1998	2373	270	0	-
1999	2513	262	0	-

The second important swordfish fishery is off Western Australia. Here domestic catches have increased from about 25 t in 1997 to 238 t in 1998 and 933 t in 1999 (R. Campbell, pers. com.).

The rapid expansion of the fisheries in the eastern AFZ and in the western AFZ mirror rapid expansion in other parts of the world. An examination of five such fisheries (Ward and Elscot 2000) showed that catches peaked and then declined, while effort continued to climb after catches peaked. Three of these fisheries appear to have been fished at unsustainable levels. Sustainable management of the Australian fisheries is required, and knowledge of stock structure is critical for management and assessment. If fish from two areas form part of the same stock, then fishing either area will impact on fish in the other area. On the other hand, if fish from the two areas form separate stocks, then fishing either area will have minimal impact on the other area. In the former instance, management should apply a single quota (for example) to the unit stock, while in the latter instance, depending on biomass and other factors, different quotas are likely to apply to the two stocks.

In practise, such a clear-cut distinction between scenarios is not possible. The degree of gene flow between two areas can be anything from zero percent to complete mixture (panmixia). It is particularly difficult to determine stock structure for marine species, as they tend to inhabit zones with no clear-cut boundaries (unlike, for example, lake or river dwelling fish). Furthermore, they often have eggs or larvae that can be distributed in currents, and adult stages are also frequently highly mobile. Only a few individuals need to migrate and interbreed to maintain genetic mixing and minimise genetic heterogeneity between areas.

The biology of swordfish might be expected to minimise stock separation. Swordfish are found in all oceans and most seas, from tropical regions to temperate regions (roughly contained by the 13°C sea surface isotherm, Nakamura 1985), and can tolerate a much broader temperature range than other billfish or tunas. Generally, juveniles are most abundant in tropical and subtropical waters; adults are thought to move to temperate waters for feeding during summer, then move to warmer waters for spawning. Adults are amongst the fastest known swimmers (up to 140 km/h, Riedl 1983).

Tagging data for swordfish are limited, and mostly pertain to the Atlantic Ocean. The farthest recorded movement currently is 2700 nautical miles across the North Atlantic in 390 days (Pepperell 2000). Other individuals have been identified as travelling from the west coast of Africa to the Azores, and from the Azores to the western Sargasso Sea (Mejuto 1991). In the Pacific, a swordfish tagged north-east of Hawaii was recaptured 3000 km away off California (D. Holts, in Rosel and Block 1996). However, most recaptures of tagged swordfish are near the point of release (Carey and Robinson 1981). Furthermore, tagging data do not provide information on gene flow; they tell you where a fish is and where it has been, but not where it reproduced.

Swordfish do not appear to have discrete spawning grounds or seasons (Grall et al. 1983), but spawn in waters at more than 20°C (Palko et al. 1981), year-round in equatorial waters but progressively limited to spring-summer at higher latitudes. Swordfish rarely gather in schools and fertilisation probably involves the pairing of otherwise solitary male and female fish (Palko et al. 1981).

Growth is initially extremely rapid, and by one year of age an swordfish can reach 90 cm and 15 kg (Ehrhardt 1992). After two to three years, females grow faster than males (Kume and Joseph 1969). Swordfish live for nine or more years (Palko et al 1981). Average maximum size for males and females predicted from models is 190–217 cm and 246–364 cm respectively (see Ward and Elscot 2000), reached at about nine and fifteen years of age respectively (Wilson and Dean 1983).

1.2. Genetic studies on swordfish

There have been several genetic studies which have examined the population structure of swordfish in various parts of the world (Table 2), but none focusing on fish in Australian waters. Most of the work to date has used the mitochondrial DNA (mtDNA) genome.

MtDNA has become widely used in the last few years for genetic studies of fish populations, as it is a rapidly evolving marker that is anticipated to be more sensitive to gene flow restrictions than nuclear DNA markers. The latter property reflects its inheritance from only the maternal parent, and its haploid nature. The genetically effective population size of mtDNA is thus, other factors being equal, only one quarter that of nDNA (which is inherited from both parents, and is diploid in each parent). This makes it potentially a more sensitive indicator of genetic drift than nDNA. The major disadvantage of mtDNA is that it is a non-recombining molecule and only provides a single estimate of population structure; nuclear DNA on the other hand provides (in principle) a vast number of largely independent markers that can be examined.

This work showed that swordfish are comprised of two main phylogenetic clades (Kotoulas et al. 1995, Alvarado Bremer et al. 1995, 1996, Rosel and Block 1996, Chow et al. 1997, Chow and Takeyama 2000). Clade I is found throughout the distribution of the species range while Clade II is found in highest frequency in the Mediterranean Sea. The most clearly, consistently and strikingly differentiated stock was that in the Mediterranean and early studies concluded that swordfish are geographically subdivided on ocean basin scales (Alvarado Bremer et al. 1995, 1996, Rosel and Block 1996, Chow et al. 1997).

Initial studies failed to reveal any mtDNA population heterogeneity of swordfish within the Atlantic Ocean (Alvarado Bremer et al. 1995, Rosel and Block 1996), but as sample sizes increased a significant north-west/south Atlantic distinction was noticed (Alvarado Bremer et al. 1996, Chow et al. 1997). However, the largest mtDNA survey failed to detect significant Atlantic heterogeneity, but concurrent study of a nDNA marker (*CaM*, calmodulin intron 4) did show a clear distinction between north-west Atlantic fish and those from tropical and southern regions (Chow and Takeyama 2000). The evidence thus favours genetic stock heterogeneity within the Atlantic.

Pacific Ocean samples of swordfish show small but significant genetic differences from Atlantic fish (Rosel and Block 1996, Alvarado Bremer et al. 1996, Chow et al. 1997, Chow and Takeyama 2000). Within the Pacific Ocean, the only allozyme examination of swordfish stock structure carried out in any ocean showed small but significant differences between north-central (Hawaii) and north-east (Mexico) samples (Grijalva-Chon et al. 1996). Another examination of nDNA variability in the Pacific, using the *CaM* locus, lacked resolving power, as the locus was monomorphic in Japanese and Peruvian samples (Chow and Takeyama 2000). MtDNA studies did not reveal any significant intra-Pacific heterogeneity (Grijalva-Chon et al. 1994, Rosel and Block 1996, Chow et al. 1997, Chow and Takeyama 2000) until a more detailed examination was carried out (Reeb et al. 2000). The latter showed patterns of mtDNA heterogeneity and homogeneity best explained by invoking a \supset shaped pattern of gene flow, such that the south-west Pacific populations (Australia) were linked to the south-east populations (Chile) and north-east populations (Mexico), which were in turn linked to north-central (Hawaii) and north-west (Japan) populations. In brief, both allozyme and mtDNA data point towards population heterogeneity in the Pacific.

Studies of Indian Ocean swordfish are limited, and insufficient to examine the possibility of intra-ocean homogeneity. Indian Ocean swordfish appear to be genetically very similar to Pacific fish (Chow et al. 1997, Chow and Takeyama 2000).

Sequencing studies of the mtDNA control region showed that the numerous observed haplotypes fell into one of two highly divergent clades, with a net nucleotide difference of about 3.9%, and with different geographic distributions (Table 2). Presumably these two divergent clades represent the remnants of a past vicariance event separating the swordfish gene pool into two isolated and independently evolving populations.

Table 2. Frequencies of the two mtDNA clades in various oceans. This excludes Chow et al. (1997) and Chow and Takeyama (2000) who did not ascribe their swordfish mtDNA haplotypes to clades.

Location	Clade		n	Reference
	I	II		
N. Atlantic	0.81	0.19	122	Alvarado Bremer et al. 1996
S. Atlantic	0.96	0.04	23	
Mediterranean	0.62	0.38	76	
N. Pacific	1.00	-	26	
N & S Atlantic	0.79	0.21	34	Rosel and Block 1996
Mediterranean	0.50	0.50	20	
E, C & W Pacific	1.00	-	105	
Indian	1.00	-	36	Reeb et al. 2000
E, C & W Pacific	1.00	-	245	

All the above studies have been summarised in Table 3. This shows that the evidence thus far identifies four major genetic stocks of swordfish: Mediterranean, north-west Atlantic, tropical and south Atlantic, and Indo-Pacific (with a complex structure in the Pacific).

Table 3. Summary of past genetic work on the swordfish. Samples within parentheses cannot be readily distinguished (Pac=Pacific Ocean, Atl=Atlantic Ocean, Ind=Indian Ocean, Med=Mediterranean Sea. N=north, C=central, E=east).

Technique	n	Conclusion regarding stock structure	Ref.
mtDNA-RFLP	148	(NW-Pac, NC-Pac, NE-Pac)	a
mtDNA-RFLP	204	(S-Atl) (W-Med, C-Med, E-Med)	b
mtDNA-d loop-seq	50	(NE-Atl, NW-Atl, S-Atl, NE-Pac) (Med)	c

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mtDNA-d loop-seq	159	(NE-Pac, SE-Pac, NC-Pac, NW-Pac) (NW-Atl, S-Atl) (Med)	d
allozyme	94	(NC-Pac) (NE-Pac)	e
mtDNA-d loop-seq	112	total N = 247 (NE-Pac) (NW-Atl, NE-Atl) (S-Atl) (Med)	f
mtDNA-d loop-RFLP	135		
mtDNA-d loop-RFLP	456	(NW-Pac, NC-Pac, NE-Pac, SE-Pac, SC-Pac, C-Ind, E-Ind, S-Atl) (NW-Atl) (Med)	g
mtDNA-d loop-RFLP	698	(Ind, Pac) (NW-Atl, C-Atl, S-Atl) (Med)	h
nDNA- <i>CaM</i>	567	(Ind, NW-Pac, SE-Pac) (C-Atl, S-Atl) (NW-Atl, Med)	
mtDNA-d loop-seq	281	*NW-Pac–NC-Pac–NE-Pac–SE-Pac–SC-Pac–SW-Pac	i

* A \supset shaped distribution was hypothesised. See text.

a, Grijalva-Chon et al. 1994; b, Kotoulas et al. 1995; c, Alvarado Bremer et al. 1995; d, Rosel and Block 1996; e, Grijalva-Chon et al. 1996; f, Alvarado Bremer et al. 1996; g, Chow et al. 1997; h, Chow and Takeyama 2000; i, Reeb et al. 2000

The present project was aimed at collecting genetic data from swordfish from the Australian east and west coast fisheries, and seeing how they related to other Indo-Pacific collections of swordfish. Carol Reeb and Barbara Block at Hopkins Marine Station, Stanford University, had already collected extensive genetic data on Pacific swordfish, and the collaboration between CSIRO Marine Research and Hopkins allowed Carol to collect genetic data on our new collections of swordfish, using her already proven methods, and to compare the new data with her pre-existing data. The Stanford laboratory obtained samples from the western Indian Ocean. A comparison of this data with fish taken off Western Australia would allow the first assessment of whether there is stock heterogeneity within the Indian Ocean.

We decided to combine studies of both mtDNA variation – as has been used in most earlier studies of swordfish stock structure (Table 3) – and the newer and potentially more powerful techniques of DNA microsatellite analysis. Microsatellites provide an abundant supply of hypervariable codominant markers for fish studies (O'Connell and Wright 1997). Both approaches were already in use in the Stanford laboratory for swordfish assessments.

2 METHODS

Swordfish samples, white muscle tissue stored in ethanol, were collected off the eastern and western coasts of Australia and from off Reunion Island (off the east coast of Madagascar) (Table 4). The Reunion Island sample was provided by B. Block at Stanford.

Table 4. Swordfish samples collected for this project

Area	Date	Location	n	Collector
Eastern Australia	July 1995	30° 20'S, 154 ° 49'E	12	M. Scott
	May-July 1999	New South Wales	31	Kevin Williams
	July 1999	S. Queensland	49	Kevin Williams
Western Australia	Feb 1995	16°S, 118°E	36	A.G.
	May-July 1999	uncertain	23	Kevin Williams
	Aug 1999	28° 46'S, 114 ° 37'E	75	Erica Starling
Reunion Island	Oct-Dec 1998	54° S, 21 °E	39	François Rene
Total			265	

2.1. Genetic methodology

Total genomic DNA was extracted from muscle tissue using the Qiagen Tissue Prep kit (Qiagen Corporation) and diluted to a final concentration of 50-100 ng/ μ l.

Mitochondrial DNA was examined by PCR amplification of two fragments from the 5' and 3' hypervariable ends of the mitochondrial control region using primers described in Rosel and Block (1996) and Reeb et al. (2000). Taq polymerase with Buffer B from Promega Corporation was used. Primer sequences are listed in Table 5. Reactions were done in 50 μ l volumes and approximately 100 ng of DNA (1-5 μ l) was added. Amplification involved 33 cycles of 1 min at 91°C, 1 min at 55°C and 2 min at 72°C. Fragments were gel purified using the Wizard Prep kit (Promega Corporation) and DNA was resuspended in 22 μ l of sterile deionized H₂O. Each fragment was sequenced on a 6% polyacrylamide/urea gel (34 cm) using the Prism DNA sequencing kit, 373A autosequencer, and Sequencing Analysis software (v. 3.0) from Applied Biosystems, Inc. Sequences were aligned to those previously published in Reeb et al. (2000).

Table 5. PCR Primers for amplifying two fragments of the control region in *Xiphias gladius*. T_A is the annealing temperature

Region	Primer (5' – 3')	T _A (°C)	Total size (bp)	Size used (bp)*
Pro-L XG-5	CTACCCCTAACTCCCAAAGC ACACCAGTCTTGTAACCC	55	350	310
XG-6 12sAR	AGCGGGTAGGGGTTTCCT ATAGTGGGGTATCTAATCCCAGTT	55	450	319

*excludes unreliable sequences and sequences from part of the 12s RNA gene

Five microsatellite loci described in Reeb et al. (submitted, also see Table 6) were amplified with PCR using 10-50 ng of DNA per μl , AmpliTaq Gold with GeneAmp 10x buffer (Perkin Elmer Corporation) and 1.5mM MgCl_2 . The DNA was then combined with one μl of GeneScan500-TAMARA loading buffer (Perkin Elmer Corporation) and electrophoresed on a 6% polyacrylamide/urea gel (24 cm) using a 373A autosequencer. The software GeneScan v. 1.2 (Applied Biosystems, Inc.), was used to analyze the gel while the software Genotyper was used to identify the various alleles.

Table 6. The five *Xiphias gladius* microsatellite loci.

Locus	Clone sequence	Size range (bp)
Xg-55	(CA) ₃₅	80-176
Xg-56	(GT) ₁₆	116-154
Xg-66	(CA) ₁₁	126-144
Xg-75	(CA) ₅₃	132-290
Xg-144	(GGA) ₇	157-175

2.2. Population genetic analysis

MtDNA: Aligned mtDNA sequences (each of 632 base pairs) were entered into Arlequin, v. 2.0 (Schneider et al. 2000). Population genetic structure was inferred using an Analysis of Molecular Variance approach (AMOVA) described in Excoffier et al. (1992). This partitions the total molecular variance into within and among population components. The program was set up using the Tamura-Nei distance algorithm (Tamura and Nei 1993). An alpha setting of 0.21 and a transition/transversion ratio of 10 previously found for the swordfish (Reeb et al. 2000) were used to determine genetic distances between pairs of populations. Haplotypes were permuted among populations 10,000 times to test for a significant departure from randomization and population homogeneity. Nucleotide diversities (Nei 1987) were also calculated.

Microsatellite loci: GenePop, v 3.2 (Raymond and Rousset 1995) was used to test for significant departures from Hardy-Weinberg equilibrium using the approach of Guo and Thompson (1992). The dememorization number, number of batches, and iterations per

batch were set to 1000 in each case F_{IS} values were estimated in GenePop using Weir and Cockerham's (1984) method.

The AMOVA option in Arlequin was used for the microsatellite data to test the null hypothesis that the three populations were not significantly different from each other (Michalakis and Excoffier 1996). Genetic distances were calculated based on the number of different alleles in each sampled population using both F_{ST} and R_{ST} (Slatkin 1995) methods for the estimation. F_{ST} methods simply use allele frequencies as the input data, R_{ST} methods use both allele frequencies and the number of repeats in each allele. F_{ST} estimates the fraction of the total variance in allele frequencies that is between populations, R_{ST} estimates the fraction of the total variance in allele size (in terms of numbers of repeat units) that is between populations. Allele sizes in base pairs were converted to number of repeats by subtracting the known length of flanking sequence (Reeb et al. submitted) from the length of each microsatellite allele. Individual genotypes were permuted among populations 10,000 times to test for significance.

In GenePop, population differentiation (genic and genotypic) was tested using the method of Raymond and Rousset (1995). Default parameters were used in the tests for significance.

2 RESULTS

3.1. Mitochondrial DNA

Data are summarised in Table 7. All mtDNA haplotypes were different from all other haplotypes, an expected consequence of sequencing a hypervariable region of DNA. Hence the haplotype diversity of each collection was 1.0. Nucleotide diversity for all collections was about 0.025.

Table 7. MtDNA. Summary of within location levels of diversity

Location	n	Haplotype diversity	Nucleotide diversity	Clade frequency			
				I	II	I ²	I ³
Western Australia	130	1.000±0.001	0.0228±0.0114	0.985	0.015	0.869	0.115
Reunion Island	38	1.000±0.006	0.0235±0.0120	1.000	-	0.895	0.105
Eastern Australia	92	1.000±0.002	0.0207±0.0104	1.000	-	0.935	0.065

In the entire sample of 260 fish, only two clade II haplotypes were recorded; both in the Western Australia sample (Table 7). These appear to be the first clade II haplotypes found in the Indian Ocean. Clade II fish are distinguished by a single repeat of the motif TACA usually starting at base 11 of the sequence, whereas clade I fish have two or three contiguous repeats of this motif at this position (Alvarado Bremer et al. 1995). There were no significant differences in the frequencies of clade I and clade II among localities ($P=0.888$). Clade I was subsequently subdivided into sequences with two TACA repeats (I²) and those with three TACA repeats (I³); there were no significant differences in the frequencies of clades I², I³ and II among localities ($P=0.478$). Representative sequences of one individual of each of clades I², I³ and II are given in Appendix 1.

The extent of sequence variation (considering all nucleotides) among and within the three locations was assessed by an AMOVA (Table 8). Nearly all the variation was due to within location variation; the percentage of variation attributable to among location differences was a non-significant 0.11. The F_{ST} value is therefore only 0.0011. As expected from this result, pairwise comparisons of the three locations failed to reveal any significant differences (Table 9).

Table 8. MtDNA. AMOVA of among location differences

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P^*
among populations	2	16.7	0.01171	0.16	0.246±0.004
within populations	257	1907.0	7.4202	99.84	
Total	259	1923.6	7.4319		

*based on 10100 permutations

Table 9. MtDNA. Pairwise comparisons of the three locations showing F_{ST} (below diagonal) and P (above diagonal) values.

	Western Australia	Reunion Island	Eastern Australia
Western Australia	-	0.425±0.005	0.212±0.004
Reunion Island	-0.0010	-	0.251±0.005
Eastern Australia	0.0018	0.0030	-

Note that a contingency table approach to this sequence data is not appropriate, as virtually every haplotype is unique and a contingency analysis will therefore have no power.

3.2 *Microsatellite loci*

Allele frequencies for each of the five microsatellite loci in each of the three populations were estimated (Appendix).

Total numbers of alleles (Table 10) per locus was very variable, ranging from 9 (locus Xg-144, the only locus with a trinucleotide repeat) to 63 (locus Xg-75), with a mean per locus of 32.4. The mean number of alleles per locus was lower in the Reunion sample (16.6) than in either the Western Australia (28.4) or Eastern Australia (27.6) samples. This does not reflect any real difference in diversity between localities; rather it reflects the reduced size of the Reunion sample (39) versus Western Australia (mean of 111.2) or Eastern Australia (mean 114.0). When examining hypervariable loci, such as these microsatellites, the larger the sample size, the more alleles will be detected.

Table 10. Microsatellite loci. Allele diversity in each sample. Sample size is numbers of fish.

Locus		Western Australia	Reunion Island	Eastern Australia	Total
Xg-55	No. alleles	45	31	45	51
	Sample size	109	39	113	261
Xg-56	No. alleles	23	16	23	25
	Sample size	112	39	115	266
Xg-66	No. alleles	13	10	11	14
	Sample size	112	39	115	266
Xg-75	No. alleles	53	36	52	62
	Sample size	111	39	112	262
Xg-144	No. alleles	8	5	7	9
	Sample size	112	39	115	266

Mean	No. alleles	28.4	16.6	27.6	32.4
	Sample size	111.2	39.0	114.0	264.2

Observed heterozygosities per locus varied from around 0.50 for Xg-144 to around 0.95 for Xg-75 (Table 11). These were also the two loci with respectively the lowest and highest numbers of alleles.

Observed genotype numbers in each sample generally accorded well with Hardy-Weinberg proportions (Table 11). A total of 15 tests (5 loci x 3 samples) were carried out. At a corrected test or P value of 0.003 (derived from 0.05/15), two tests significantly deviated from expectations. Both were in the Western Australia sample (loci Xg-66 and Xg-144). These are the two least diverse loci for numbers of alleles and heterozygosity. Both showed significant homozygote excesses or heterozygote deficiencies, with F_{IS} values of around 0.18. In fact, all 15 samples showed homozygote excesses or heterozygote deficiencies, giving positive values for F_{IS} , although most were small and non-significant.

Table 11. Microsatellite loci. Probability of fit to Hardy-Weinberg expectations. Observed and expected heterozygosities and F_{IS} values (after Weir and Cockerham) also given.

Locus		Western Australia	Reunion Island	Eastern Australia
Xg-55	P of HW test	0.358±0.014	0.134±0.010	0.021±0.004
	Obs heterozygosity	0.917	0.949	0.942
	Exp heterozygosity	0.967	0.966	0.967
	F_{IS}	0.052	0.018	0.040
Xg-56	P of HW test	0.372±0.012	0.451±0.012	0.100±0.008
	Obs heterozygosity	0.839	0.821	0.843
	Exp heterozygosity	0.916	0.846	0.897
	F_{IS}	0.084	0.031	0.060
Xg-66	P of HW test	0.001±0.001	0.109±0.004	0.235±0.007
	Obs heterozygosity	0.705	0.769	0.748
	Exp heterozygosity	0.865	0.870	0.828
	F_{IS}	0.185	0.117	0.097
Xg-75	P of HW test	0.720±0.014	0.369±0.015	0.027±0.005
	Obs heterozygosity	0.946	0.949	0.929
	Exp heterozygosity	0.971	0.967	0.969
	F_{IS}	0.027	0.020	0.042
Xg-144	P of HW test	0.001±0.001	0.711±0.004	0.498±0.007
	Obs heterozygosity	0.536	0.487	0.522

Exp heterozygosity	0.647	0.526	0.606
F_{IS}	0.173	0.043	0.140

Variation among and within the three locations was assessed by an AMOVA using the Arlequin package (Table 12). Nearly all the variation was due to within location variation; however, the percentage of variation attributable to among location differences was a statistically significant 0.46 ($P=0.001$). The F_{ST} value is therefore 0.0046 (Table 12A). These differences were then investigated on a locus by locus basis to determine which locus or loci were responsible for the differentiation. This showed that locus Xg-144 was the major contributor (Table 12B), with by far the highest F_{ST} value (0.022) and the only one significantly different from zero after Bonferroni correction. Pairwise comparisons of the three locations (Table 12C) showed that the differentiation was mostly due to the Reunion Island sample, which was significantly differentiated from Western Australia. However, even in this pairwise comparison, about 99% of all variation is due to within locality variation, with about 1% ($F_{ST}=0.0113$) due to population differences.

Table 12. Microsatellite loci. Testing among location differences. AMOVA analyses (Arlequin).

(A) Across all three locations and five loci

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P^*
among populations	2	7.4	0.0098	0.46	0.001±0.001
within populations	529	1118.3	2.1140	99.54	
Total	531	1125.7	2.1237		

*based on 1023 permutations. $F_{ST} = 0.0046$ ($P=0.001$)

(B) Across all three locations, locus by locus

Locus	d.f.	F_{ST}	P
Xg-55	2, 519	-0.0017	0.963
Xg-56	2, 529	0.0033	0.086
Xg-66	2, 529	0.0065	0.044
Xg-75	2, 521	-0.0009	0.840
Xg-144	2, 529	0.0217	0.005

(C) Pairwise comparisons of the three locations showing F_{ST} (below diagonal) and P (above diagonal) values.

	Western Australia	Reunion Island	Eastern Australia
Western Australia	-	0.001±0.001	0.044±0.007

Reunion Island	0.0113	-	0.073±0.008
Eastern Australia	0.0025	0.0039	-

The extent of genic differentiation among populations was also assessed by the GenePop package (Table 13). Individual locus F_{ST} values ranged from 0.0198 (Xg-144) to -0.0020 (Xg-55), none was significant using the contingency table/Markov chain approach. These individual locus F_{ST} values were very similar to those estimated by Arlequin; the major difference lay in the P value attributed to locus Xg-144. This was a non-significant 0.077 in GenePop but a highly significant 0.005 in Arlequin. The F_{ST} value across loci was estimated at 0.0038 (similar to the 0.0046 estimated by the AMOVA), but the test combining the probability values across loci was non-significant ($P=0.279$). This contrasts with the highly significant value result for overall F_{ST} from Arlequin. There was also no significant pairwise heterogeneity between any pair of samples for any locus (after Bonferroni correction for 3x5 tests) or across loci, again contrasting with the Arlequin result.

Table 13. Microsatellite loci. Testing among location differences for individual loci. F_{ST} results and P values from contingency table/Markov chain analyses (GenePop).

(A) Across all three locations

Locus	F_{ST}	P value
Xg-55	-0.0020	0.963±0.001
Xg-56	0.0030	0.481±0.002
Xg-66	0.0053	0.081±0.001
Xg-75	-0.0012	0.826±0.002
Xg-144	0.0198	0.077±0.001

Across loci: $F_{ST} = 0.0038$

Probability (Fisher's method)

chi=12.091, df = 10, $P = 0.279$ (B) Pairwise comparisons of allele frequencies for each locus for the three locations. P values shown, from GenePop

Loci		Reunion Island	Eastern Australia
Xg-55	Western Australia	0.943±0.002	0.821±0.004
	Reunion Island		0.941±0.002
Xg-56	Western Australia	0.156±0.003	0.667±0.005
	Reunion Island		0.597±0.005
Xg-66	Western Australia	0.789±0.003	0.013±0.001
	Reunion Island		0.442±0.004
Xg-75	Western Australia	0.413±0.005	0.970±0.002
	Reunion Island		0.534±0.005
Xg-144	Western Australia	0.019±0.001	0.293±0.005
	Reunion Island		0.259±0.003
Across loci*	Western Australia	0.177	0.211
	Reunion Island		0.720

*estimated by Fisher's method by combining the individual locus values and estimating a chi-square with associated degrees of freedom

The final analysis makes use of the fact that microsatellite data not only carries frequency information but also size information (number of repeat units per allele). This is Slatkin's (1995) R_{ST} approach. The AMOVA method of Arlequin permits an R_{ST} analysis; this

showed no significant differentiation among populations (Table 14), indeed, the among-population variance component or R_{ST} value was negative.

Table 14. Microsatellite loci. Testing among location differences. R_{ST} analyses using AMOVA (Arlequin).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P^*
among populations	2	-22.135	-1.614	-0.64	0.994 \pm 0.001
within populations	529	133631.6	252.612	100.64	
Total	531	133609.4	250.998		

*based on 10100 permutations. $R_{ST} = -0.0064$.

4. DISCUSSION

4.1 Mitochondrial DNA

Examination of mtDNA sequence diversity failed to reveal any statistically significant divergence among the three locations examined. These data do not allow us to reject the null hypothesis that fish from Reunion Island, western Australia and eastern Australia form part of the same gene pool.

4.2. Microsatellite loci

All five microsatellite loci were highly variable, with from nine (Xg-144) to 62 (Xg-75) alleles per locus. Goodness of fit of observed genotype frequencies to Hardy-Weinberg expectations were generally acceptable, except in two instances: Xg-66 in Western Australia and Xg-144, also in Western Australia. In both instances there was a significant homozygote excess (heterozygote deficiency) with F_{IS} values of around 0.18 (Table 11).

Homozygote excesses can be caused by one or a combination of several factors. Chief among these are null alleles, and population admixture.

Null alleles in the microsatellite context are alleles that do not amplify; generally because of mutations in one or other of the two priming sites or because of preferential amplification of the smaller allele in a heterozygote. A heterozygote for an expressed allele and a null allele will be wrongly scored as a homozygote for the expressed allele, leading to an apparent homozygote excess. If null alleles were the explanation for the homozygote excesses in Xg-66 and Xg-144 in Western Australia, then given the genetic similarities between locations (see later), then they would be expected to be present in the other localities too. In fact, while other localities did accord with Hardy-Weinberg expectations, they too showed homozygote excesses possibly indicative of null alleles. Table 15 shows that the mean F_{IS} values for Xg-66 and Xg-144 were substantially higher than for the other three microsatellite loci, whether or not the Western Australia sample is included. This is consistent with the presence of null alleles at these two loci in all samples. If null alleles are present, maximum likelihood estimates of frequency can be determined using the GenePop package. As expected, null allele frequencies appear to be highest for loci Xg-66 and Xg-144 (Table 15). This is especially so for locus 66 in western Australia and Reunion Island while locus 144 has the highest null frequency in western Australia. Arguing against the null allele explanation is the fact that these two loci were the least variable in terms of numbers of alleles (Table 10), had the smallest size ranges of any loci (Table 6), and perhaps the clearest and easiest-to-score gels (Xg-144 was particularly simple to score being the only tri-nucleotide repeat locus examined). Since we cannot be certain that null alleles do exist in our samples, we have ignored this possibility in our population structure analyses.

Table 15. Mean F_{IS} values and estimates of null alleles.

Locus	Mean F_{IS} across populations		Null allele frequency			
	Western Australia	Reunion Island	West	Reunion	East	Mean

Population structure of Australian swordfish

	Reunion Island Eastern Australia	Eastern Australia	Australia	Island	Australia	
Xg-55	0.042	0.019	0.023	0.006	0.019	0.016
Xg-56	0.067	0.046	0.049	0.010	0.033	0.031
Xg-66	0.138	0.107	0.087	0.099	0.041	0.076
Xg-75	0.032	0.031	0.010	0.029	0.021	0.020
Xg-144	0.143	0.092	0.078	<0.001	0.055	0.044

Admixture of genetically differentiated populations will also cause a homozygote excess. This is often termed the Wahlund effect. For example, if two genetically isolated and differentiated spawning stocks that shared a common feeding ground were sampled from the feeding ground, a homozygote surplus over Hardy-Weinberg expectations would be detected. The extent of this surplus depends on the degree of differentiation of the stocks; small differentiation would result in small surplus, large differentiation in a large surplus.

The three localities sampled in this project differ little if at all (see later) for microsatellite allele frequencies, and so admixture would not be expected to result in a significant homozygote surplus. Thus the Wahlund effect seems an unlikely explanation for the positive F_{IS} values. On the other hand, the two loci with the highest F_{IS} values (Xg-144 and Xg-66) also have the highest F_{ST} values across populations (Table 16), consistent with the hypothesis that at least part of the homozygote excess at these two loci might be attributed to population heterogeneity.

Table 16. F_{ST} and F_{IS} values for each locus across the three populations (GenePop).

Locus	F_{ST}	F_{IS}
Xg-55	-0.0020	0.0416
Xg-56	0.0030	0.0666
Xg-66	0.0053	0.1377
Xg-75	-0.0012	0.0317
Xg-144	0.0198	0.1433

At present, the explanation for the small homozygote excesses at the five microsatellite loci – statistically significant for two loci – remains uncertain. The relative contributions made by null alleles, population heterogeneity, and other factors which might include genotyping errors, cannot be assessed. However, whatever the explanation, the fact remains that most population samples were in Hardy-Weinberg equilibrium. The effect of these small homozygote excesses on any conclusions about population structure is therefore likely to be negligible.

Is there significant differentiation in microsatellite loci among the three localities sampled? Several different statistical analyses of the data were carried out, with partially conflicting results.

The AMOVA analyses of allele frequencies (Table 12) did show significant differentiation among the three localities ($P=0.001$), although the amount of variation that could be attributed to population differentiation was very small, less than 1% ($F_{ST}=0.0046$). Much of this was due to locus Xg-144 and to (minor) differentiation of the Western Australian population from the others

On the other hand, the contingency table analyses of allele frequencies (Table 13) failed to show any significant differentiation among the three localities, for any individual locus or across the five loci or among pairwise population comparisons. Interestingly, the resulting F_{ST} estimates from GenePop, for individual loci and across loci, were in fact very similar to the AMOVA estimates from Arlequin; only the P values varied. In GenePop, construction of contingency tables through resampling methods produced null distributions which were tested using a chi square analysis. Arlequin used permutation and resampling procedures and F statistics. Locus Xg-144 in particular was deemed to be non-significantly differentiated by GenePop ($F_{ST}=0.0198$, $P=0.077$) but very significantly differentiated by Arlequin ($F_{ST}=0.0217$, $P=0.005$). The order of magnitude difference in P values is likely attributable to the fact that the conventional chi square test employed in Monte Carlo randomization methods implemented in GenePop is conservative (Lewontin and Felsenstein 1965, Sokal and Rohlf 1995, pg 810) and thus divergences must be large to show significant differences between populations of small sample size. Hence, the probability of a Type I error will be lower in GenePop than Arlequin. In both methods, accuracy of the statistical tests depends on sample size and the number of permutations used to generate the null distributions.

The AMOVA analysis of microsatellite repeat number variation (Table 14) failed to show any significant differentiation among the three localities. Indeed, the estimated amount of variation that could be attributed to population differentiation was slightly negative ($R_{ST}=-0.0064$, non-significant).

Thus, one statistical test revealed significant (if small) microsatellite differentiation among populations but others found non-significant differences. Had there been sizeable differentiation, then all statistical approaches would have been expected to show it. The evidence for differentiation at microsatellite loci among these three localities is therefore weak and may require still larger sample sizes to conclusively support differentiation among the three populations sampled in this study. However, an interesting trend is apparent. Both GenePop and Arlequin show that comparisons between western Australia and either Reunion Island or East Australia results in lower P values than comparisons between these latter two populations.

4.3 Combining mtDNA and microsatellite data

The mtDNA data showed no statistically significant evidence of differentiation among the three localities. The microsatellite data were somewhat equivocal but indicated that differentiation, if any, was very limited. The suggestion from the microsatellite data that Western Australia might be a little different from Reunion Island and Eastern Australia was not supported by the mtDNA data. While it is tempting to speculate that Reunion Island might be different from Australian populations by its proximity to the Atlantic Ocean (Atlantic Ocean swordfish are known to be differentiated from IndoPacific swordfish for both mtDNA and microsatellites (Alvarado Bremer et al 1996, Chow and Takeyama 2000, Rosel and Block 1996, Reeb unpublished), and might receive some immigrants from the Atlantic, we have no hard evidence that this is in fact the case. Indeed, the presence of two mtDNA clade II fish in the Western Australia sample and their absence from the Reunion sample argues against this explanation. Unfortunately, the sample size from Reunion was only 38 compared with closer to 100 for each of the Western and Eastern Australia localities so the rare Clade II haplotypes were likely missed.

The genetic data on swordfish in the eastern and western Australian fishing zones fail to show strong evidence of population differentiation. There may be a single stock in this area, possibly connected by gene flow around Tasmania and southern Australia. Arguing against this is the slight microsatellite differentiation of Western Australia. In addition, the presence of two mtDNA clade II fish in the Western Australia sample and its absence from all Pacific Ocean fish examined to date is tantalizing evidence of possible Atlantic input into the Indian Ocean swordfish population but not into the Pacific population. Indian Ocean swordfish are believed to move with the warm Agulhas current that sweeps around South Africa into the Atlantic Ocean (Penny and Griffiths 1998), and maybe there is some reciprocal movement too.

4.4 Indo-Pacific swordfish population structure

It is clear that the West Australia samples we examined from the Indian Ocean were genetically very similar to East Australia samples from the Pacific Ocean, for both mtDNA and microsatellite loci. These data were put into a broader context by comparing them with mtDNA and microsatellite variation in other samples analysed (Reeb et al. 2000, and Reeb, unpublished) from the Pacific Ocean (Chile, Ecuador, Japan) and the Atlantic Ocean (Brazil).

An earlier examination of mtDNA sequence diversity had revealed evidence of a \supset shaped corridor of gene flow within the Pacific Ocean (Reeb et al. 2000). Chilean and Japanese populations appeared to be genetically distinct with ranges that overlapped in the eastern Pacific. The East Australia sample examined here was not different from the Chilean sample ($P=0.315$) but was distinct from the Japanese sample ($P=0.014$). This then adds to the evidence of different northern and southern hemisphere stocks of swordfish in the Pacific.

It is noteworthy that all but two fish of the fish sampled in the present project were mtDNA clade I, and that the two clade II fish were both from the Western Australia sample. Available data on the distribution of these two clades, by ocean (Table 17), show that these

are the only clade II fish so far found in the Indian Ocean and no clade II fish have been detected in the Pacific Ocean. While this difference is not statistically significant ($P=0.186$), the presence of clade II fish at low levels in the Indian Ocean and at quite high frequency in the Atlantic Ocean (around 0.17, perhaps less in the South Atlantic - see Table 2), might suggest some limited gene flow from the Atlantic into the Indian Ocean but not into the Pacific Ocean. This gene flow could be current or historical. Reunion Island is far closer to the Atlantic than is Western Australia, and the absence of clade II fish at Reunion might simply reflect its small sample size (38 vs. 130). Chow and Takeyama (2000), while not indicating whether any clade II fish were present, failed to show any significant differences in restriction fragment patterns of the mtDNA d-loop from 84 Indian Ocean and 236 Pacific Ocean fish. Thus the clade I versus II data do not allow us to reject the null hypothesis of a single Indo-Pacific stock.

Table 17. Summary of the distribution of mtDNA clade I and clade II fish.

Ocean/Sea	Clade		n	Reference
	I	II		
Pacific	1.000 (351)	0.000 (0)	351	see below
Indian	0.988 (128)	0.012 (2)	168	see below
Atlantic	0.827 (148)	0.173 (31)	179	see below
Mediterranean	0.594 (57)	0.406 (39)	96	see below
Data sources:				
Pacific	26	0	26	Alvarado Bremer et al. 1996
Pacific	245	0	245	Reeb et al. 2000
Pacific	80	0	80	this study ¹
Indian	36	0	36	Reeb et al. 2000
Indian	130	2	132	this study ²
Atlantic	121	24	122	Alvarado Bremer et al. 1996
Atlantic	27	7	34	Rosel and Block 1996
Mediterranean	47	29	76	Alvarado Bremer et al. 1996
Mediterranean	10	10	20	Rosel and Block 1996

¹excludes 12 eastern Australia samples already included in Reeb et al. 2000

²excludes 36 western Australia samples already included in Reeb et al. 2000

To summarise, the mtDNA data suggest that there is a northern Pacific swordfish stock and a southern Pacific swordfish stock, overlapping in the central east Pacific, and that southern Pacific stock swordfish cannot at present be separated from Indian Ocean swordfish (Table 18). This result was obtained by Reeb et al. (2000) and is again observed here.

Table 18. Mitochondrial DNA Sequence. Testing among group differences. F_{ST} analysis. Groups are (Reunion Island, East Australia, West Australia), (Chile/), (Japan, Hawaii, California/Mexico) AMOVA analyses (Arlequin).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
among groups	2	31.588	0.06626	0.90
among populations within groups	4	31.563	0.01049	0.14
within populations	432	3130.567	7.24668	98.95
Total	438	3193.718	7.32344	

Source of variation		Fixation index	<i>P</i> value
among groups	F_{CT}	0.0091	0.017
among populations within groups	F_{SC}	0.0015	0.258
among populations	F_{ST}	0.0105	0.001

This conclusion of at least two separable stocks is different from the one that Chow and Takeyama (2000) reached. They reported no mtDNA genetic differentiation between Indian Ocean samples (total $n = 84$, from three regions: tropical north east, tropical southeast, tropical west) and Pacific Ocean samples (total $n=236$, six regions: northwest, tropical central north, northeast, tropical east, southeast, southwest). It may be that the RFLP approach they took (rather similar to clade I vs clade II) for mtDNA analysis was not sufficiently sensitive to detect the limited differentiation we observe following DNA sequencing.

For the first microsatellite Indo-Pacific analysis, the Reunion Island and East and West Australia samples were pooled, and compared with Chile and Ecuador (pooled), and Japan giving three groups. An AMOVA-based F_{ST} analysis (data not shown) revealed significant differences among the five populations ($P = 0.0048$, Chile and Ecuador remaining pooled), no significant differences among groups ($P = 0.5993$), and a significant difference among populations within groups ($P = 0.0034$). This suggests that the Indo-Pacific may comprise more than one stock. However, an R_{ST} analysis of the data, again carried out as an AMOVA in Arlequin, showed no significant differentiation (data not shown).

The conclusion from the microsatellite analysis is that clear statistical support for stock structure in the Indo-Pacific is limited, but there is some evidence of stock heterogeneity, especially in the Indian Ocean with the western Australian population as noted earlier. Chow and Takeyama (2000) found no evidence of differentiation among Indo-Pacific samples for another nuclear DNA locus (calmodulin gene intron 4, *CaM*).

Combing the mtDNA and nuclear DNA evidence suggests several stocks: (1) a northern Pacific stock (differentiated by mtDNA), (2) a southern Pacific stock (differentiated by mtDNA) which is, surprisingly, genetically similar to Reunion Island, and (3) a western Australia stock (differentiated by microsatellite DNA).

The western Australia differentiation is unexpected. The Indonesian throughflow, a net flow of surface water from the southern extremity of the Asian mainland through the Indonesian archipelago to north-west Australia (e.g. Meyers et al 1995, Gordon and Fine 1996, Schiller et al 1998), may well transport swordfish larvae and juveniles from the western tropical Pacific to the north-east Indian Ocean. The western tropical Pacific is an important spawning ground of swordfish, and high concentrations of larvae have been reported in the north-east but not western parts of the Indian Ocean (Nishikawa et al 1985). Adult movements along the southern coasts of Australia and Tasmania might also have been expected to lead to gene flow between the eastern Pacific and western Indian Ocean. Records of Japanese long liners show that appreciable numbers of swordfish have been taken from off South Australia (R. Campbell, pers. com.).

The stock structure of Australia's swordfish resource - whether Australia's fished populations represent distinct stocks or are parts of much larger stocks - is critical for management (Ward and Elscot 2000, page 149). However, our study was unable to unequivocally resolve this issue. If there are distinct stocks, then the degree of separation of them is small. A larger study of the genetics of swordfish of this area is required.

A tagging study of the eastern and western populations would provide valuable data. Such data are not available for Indian or Pacific Ocean populations. This may at least partly reflect the difficulty of such studies for swordfish, as the fish are relatively scarce and very valuable. In addition, swordfish obtained on longlines are usually dead or moribund when lines are retrieved, and those retrieved alive are aggressive and difficult to tag (Ward and Elscot 2000, page 126).

4.5 Future research

We recommend a larger scale examination of this topic. Some suggestions of stock structure were seen in the present data (which met the sample sizes proposed in the project outline), including the possible differentiation of western Australia, but they need to be confirmed or rejected from a larger data set. Either outcome has important implications for management. We would recommend that sample sizes be increased to a minimum sample size of 200 from each of eastern and western Australia, and that at least three additional regions be examined in the Indian Ocean. It would also be beneficial to increase the number of microsatellite loci from five to eight. An analysis of mtDNA should also form part of the proposed project. We recommend that this take the form of sequencing as performed herein; a restriction length polymorphism approach is likely to lack adequate power.

ACKNOWLEDGEMENTS

This project was largely funded by the Australian Fisheries Management Authority (project R99/0159). Funding for this project was also provided by Cooperative Agreement Number NA37RJ0199 from the National Oceanic and Atmospheric Administration (NOAA). The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its subagencies. We are very grateful to François Rene, Erica Starling and Kevin Williams for supplying swordfish tissue samples for this project. Shana Beemer provided excellent technical assistance in DNA extractions and sequencing

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APPENDIX 1. Representative sequences of mtDNA clades I2, I3 and II

Clade I2

Fish East Australia 99 SW09

```

1 CA--TTTTT- AAA-TACATA CA----- TGTATGTATT AACACCATAC
51 ACTTATATTA ACCATATAAT CCAATGTTTC AGCACATAAT ATGTAATTCG
101 ACCATTA-AT AACAATGTAA CCATTCATAC TTATACAATA ATAATAAGGT
151 AGACATAAAC CATAATTTTG TAGCTTCAAT AAGACACTTA G-T-TAAACA
201 AG-CAGAAAC TTAAGACCTA GCACTTTAAA TTAATGTGTC TAGTTATACC
251 AGGACTCAA TTCCGATTGA ACCCAGAATC TTAATGTAGT AAGAGACCAC
301 CAACCAGTAT ATTTTACxxT CACTTGACAT T-TCAGAGTG CACACGGTAT
351 AATTAANAATA AGGTTGTACA TTTCCTTGCT TGG-AAGAAA ATTTATTAAA
401 GGTGGAAAGA CTTTACACAA GAAA-TACAT ACTTGGATAT CAAGGACATA
451 AAGTATTTGT TCTTCTCCTA AAATACCTAA GATACCCCC ---GTTTTTG
501 CGCGTAAAAC CCCC-TACC CCCAAAACCT CCAgAGATCA CTAACACTCC
551 TGA AACCCC CCGGAAACAG GAAAACCTCT AGCAGCTTCT TTTTACCTCA
601 AAATGCGTCT ATTTTACTA TTAAAATATT TCACATGC

```

Clade I3

Fish East Australia 99 SW14

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1 TA--TTTTT- AAA-TACATA CATA----- TGTATGTATT AACACCATAC
51 ACTTATATTA ACCATATAAT CCAATGTTTT AGCACATAAT ATGTAATTCa
101 ACCATTA-AT AACAATGTAA CCATTCATAC TTATACAACA ATAATAAGGT
151 AGACATAAAC CATAATTTTG TAACCTCAAT AAGACACTTA A-T-TAAACA
201 Ag-CAGAAAC TTAAGACCTA GCACTTtAAA TTAATGTGTC TAGTTATACC
251 AGGACTCAA TTCCGATTGA ACCCAGAATC TTAATGTAGT AAGAGACCAC
301 CAACCAGCAT ATTTTACxxT CACTtGACAT T-TCAGAGTG CACACGGTAT
351 AATTAANAATA AGGTTGTACA TTTCCTTGCT TGG-AAGAAA ATTTATTAAA
401 GGTGGAAAGA CTTTACACAA GAGA-TACAT ATTTGGATAT CAAGGACATA
451 AAGTATTTGT TCTTCTCCTA AAATACCTAA GATACCCCC ---GTTTTTG
501 CGCGTAAAAC CCCC-TaCC CCCAAAACCT CCAGAGATCA CTAACACTCC
551 TGA AACCCC CCGGAAACAG GAAAACCTCT AGCAGCTTCT TTTTACCTCA
601 AAATGCGTCT ATTTTACTA TTAAAATATT TCACATGC

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Clade II

Fish West Australia 99 SW123

```

1 CA--TTTTT- AAA-TACA-- ----- TGTATGTATT AACACCATAC
51 ATTTATATTA ACCATATAAT CCAATGTTTT AGTGCATAAT ATGTAACTCG
101 ACCATT-TAT AACAATGTAA CCATTCGTGC TCACACAACA ATAATAAGGT
151 AGACATAAAC CATAGCCTTG TAACCTCAAT AAGACATTTA A-TCTAAACA
201 AA-CAGAAAC TTAAGACCTA ACACTTCAAA TTAATGTGTC TAGTTATACC
251 AGGACTCAA TTCCGGTTGA ACTCCAGAAT CCTTAATGTA GTAAGAGACC
301 ACCAACCAGC ATATCTCxxT CaCTTGACaT T-TCAGAGTG CACACGGTAT
351 AACTANAATA AGGTTGTACA TTTCCTTGCT TGGGAAGAAA ATCTATTAAA
401 GGTGGAAAGA CTTTACACAA GAAA-TACAT ACTTGGATAT CAAGGACATA
451 AAGTATTTGT TCTTCTCCTA AAATACCTAA gATACCCCC ---GTTTTTG
501 CGCGTAAAAC CCCC-TACC CCCAAAACCT CCAgAgATCA CTAACACTCC
551 TGA AACCCC CCGGAAACAG GAAAACCTCT AGCAGCTTCT TTTTACCTCA
601 AAATGCGTCT ATTTTACTA TTAAAATATT TCACATGC

```

APPENDIX 2. Allele frequencies for five microsatellite loci in swordfish from three localities in the Indo-Pacific. Also given are observed and Hardy-Weinberg expected heterozygosities, and *P* values for goodness-of-fit to Hardy-Weinberg equilibrium.

Xg- 55

Allele Size	Repeats	West Australia	Reunion Island	East Australia
88	1	-	-	0.0133
90	2	0.0046	-	-
96	5	0.0046	-	-
98	6	0.0046	-	0.0044
100	7	0.0321	-	0.0133
102	8	0.0046	0.0128	0.0088
104	9	0.0046	0.0256	0.0133
106	10	0.0275	0.0128	0.0177
108	11	0.0229	0.0128	0.0221
110	12	0.0275	0.0641	0.0442
112	13	0.0505	0.0513	0.0442
114	14	0.0505	0.0769	0.0664
116	15	0.0505	0.0385	0.0442
118	16	0.0413	0.0897	0.0619
120	17	0.0550	0.0641	0.0310
122	18	0.0505	0.0385	0.0619
124	19	0.0550	0.0513	0.0619
126	20	0.0596	0.0385	0.0265
128	21	0.0413	0.0513	0.0398
130	22	0.0183	0.0128	0.0487
132	23	0.0275	0.0128	0.0354
134	24	0.0505	0.0385	0.0354
136	25	0.0367	0.0385	0.0354
138	26	0.0275	0.0128	0.0221
140	27	0.0229	0.0513	0.0177
142	28	0.0184	0.0256	0.0310
144	29	0.0321	0.0256	0.0177
146	30	0.0137	0.0256	0.0133
148	31	0.0321	-	0.0133
150	32	0.0046	-	0.0133
152	33	0.0321	0.0256	0.0044
154	34	0.0092	-	0.0044
156	35	0.0046	0.0128	0.0088
158	36	0.0092	0.0256	0.0088
160	37	0.0046	-	0.0133
162	38	0.0092	-	0.0265
164	39	0.0092	0.0128	0.0088
166	40	0.0046	-	0.0088

Population structure of Australian swordfish

168	41	0.0046	-	0.0133
170	42	0.0092	0.0128	0.0044
174	44	-	-	0.0088
176	45	0.0046	-	0.0044
178	46	-	-	0.0044
180	47	0.0046	0.0128	0.0044
184	49	0.0092	-	0.0088
188	51	0.0046	-	-
190	52	-	0.0128	-
196	55	0.0046	-	0.0044
200	57	-	-	0.0044
202	58	-	0.0128	-
204	59	0.0046	-	-
n		109	39	113
Obs.Heter.		0.917	0.949	0.942
Exp.Heter		0.967	0.966	0.967
<i>P. value</i>		0.358	0.134	0.021

Xg-56

Allele Size	Repeats	West Australia	Reunion Island	East Australia
108	2	0.0045	-	-
120	8	0.0402	0.0128	0.0304
122	9	0.0491	0.0641	0.0696
124	10	0.0446	0.0128	0.0217
126	11	0.0268	0.0128	0.0087
128	12	0.1384	0.0641	0.1217
130	13	0.0223	0.0128	0.0304
132	14	0.0536	0.1154	0.0565
134	15	0.1920	0.3333	0.2435
136	16	0.0714	0.1410	0.0870
138	17	0.0670	0.0513	0.0565
140	18	0.0491	0.0128	0.0305
142	19	0.0402	0.0128	0.0652
144	20	0.0714	0.0769	0.0652
146	21	0.0223	0.0385	0.0087
148	22	0.0089	0.0256	0.0304
150	23	0.0402	0.0128	0.0261
152	24	0.0089	-	0.0087
154	25	0.0179	-	0.0043
156	26	0.0179	-	0.0130
162	29	-	-	0.0043
164	30	0.0045	-	0.0043
166	31	0.0045	-	0.0087

Population structure of Australian swordfish

168	32	0.0045	-	-
170	33	-	-	0.0043
n		112	39	115
Obs.Heter.		0.839	0.821	0.843
Exp.Heter		0.916	0.846	0.897
<i>P</i> value		0.372	0.451	0.100

Population structure of Australian swordfish

Xg-66

Allele Size	Repeats	West Australia	Reunion Island	East Australia
126	8	0.0759	0.1154	0.0696
128	9	0.0179	0.0128	0.0043
130	10	0.0223	0.0256	0.0217
132	11	0.1518	0.1154	0.0565
134	12	0.1786	0.2308	0.3087
136	13	0.1875	0.1795	0.1826
138	14	0.1250	0.0897	0.1087
140	15	0.1473	0.1154	0.1391
142	16	0.0670	0.0641	0.0870
144	17	0.0134	0.0513	0.0174
146	18	-	-	0.0043
148	19	0.0045	-	-
154	22	0.0045	-	-
160	25	0.0045	-	-
n		112	39	115
Obs.Heter.		0.705	0.769	0.748
Exp.Heter		0.865	0.870	0.828
<i>P</i> value		0.0007*	0.109	0.235

Xg-75

Allele Size	Repeats	West Australia	Reunion Island	East Australia
134	21	-	-	0.0045
138	23	-	0.0128	-
148	28	-	-	0.0045
152	30	0.0045	-	-
158	33	0.0045	-	0.0089
164	36	0.0045	-	0.0089
166	37	0.0180	0.0128	0.0179
168	38	0.0045	-	-
170	39	0.0090	0.0128	-
172	40	0.0045	0.0256	0.0045
174	41	0.0135	-	0.0089
176	42	0.0270	0.0256	0.0045
178	43	0.0135	0.0128	0.0134
180	44	0.0360	0.0128	0.0357
182	45	0.0315	0.0128	0.0402
184	46	0.0270	0.0256	0.0268
186	47	0.0541	0.0897	0.0491
188	48	0.0405	0.0641	0.0268
190	49	0.0450	0.0128	0.0491

Population structure of Australian swordfish

192	50	0.0631	0.0385	0.0580
194	51	0.0405	0.0513	0.0670
196	52	0.0405	0.0769	0.0580
198	53	0.0405	0.0769	0.0402
200	54	0.0631	0.0256	0.0491
202	55	0.0541	0.0769	0.0491
204	56	0.0360	0.0256	0.0402
206	57	0.0045	0.0385	0.0089
208	58	0.0045	-	0.0089
210	59	0.0270	0.0128	0.0134
212	60	0.0270	-	0.0179
214	61	0.0315	0.0128	0.0179
216	62	0.0180	0.0256	0.0268
218	63	0.0180	-	0.0089
220	64	0.0090	0.0128	0.0179
222	65	0.0045	0.0256	0.0045
224	66	0.0090	0.0128	0.0223
226	67	0.0135	0.0128	0.0446
228	68	0.0090	-	0.0223
230	69	0.0270	0.0128	0.0045
232	70	0.0090	-	0.0134
234	71	0.0135	0.0128	0.0089
236	72	0.0135	0.0128	0.0134
238	73	0.0045	-	0.0045
240	74	0.0225	-	0.0089
242	75	0.0090	-	0.0045
244	76	0.0045	-	0.0045
246	77	0.0045	-	0.0045
248	78	0.0045	0.0256	0.0089
250	79	-	0.0256	0.0089
252	80	-	0.0128	0.0045
254	81	0.0045	0.0128	0.0089
256	82	0.0045	-	-
260	84	-	-	0.0045
262	85	0.0045	-	0.0045
264	86	-	-	0.0045
266	87	0.0090	-	-
270	89	0.0045	-	0.0045
272	90	0.0045	-	-
274	91	0.0045	-	-
278	93	-	0.0128	0.0045
282	95	-	0.0256	-
290	99	0.0045	-	-
n		111	39	112
Obs.Heter.		0.946	0.949	0.929
Exp.Heter		0.971	0.967	0.969

Population structure of Australian swordfish

P value 0.720 0.369 0.027

Xg-144

Allele Size	Repeats	West Australia	Reunion Island	East Australia
154	9	0.0089	-	-
157	10	0.0268	0.0128	0.0174
160	11	0.4063	0.6538	0.5000
163	12	0.4196	0.2564	0.3696
166	13	0.1161	0.0641	0.0957
169	14	0.0045	-	0.0087
172	15	0.0089	0.0128	0.0043
175	16	0.0089	-	-
178	17	-	-	0.0043
n		112	39	115
Obs.Heter.		0.536	0.487	0.522
Exp.Heter		0.647	0.526	0.606
<i>P</i> value		0.001*	0.711	0.498

* Significant *P* values after Bonferroni correction