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Characterisation of 22 polymorphic microsatellite loci for the Broadbill Swordfish, *Xiphias gladius*

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Abstract :

The Broadbill Swordfish is harvested by fisheries throughout the world. In order to devise more effective management strategies, we need a clear understanding of the population structure of the species. From a library of 29 tetranucleotide repeats, 22 microsatellite markers were characterised for 94 swordfish samples captured from eastern and western Australia. The number of alleles ranged from 2 to 26 and observed heterozygosity from 0.066 to 0.923. We have identified 18 microsatellites that will be valuable in the examination of swordfish population structure.

Keywords : Swordfish – *Xiphias gladius* – Microsatellite – Genetic markers

The Broadbill Swordfish, *Xiphias gladius*, is a large pelagic fish species harvested by fisheries worldwide. Although swordfish are capable of migrating long distances, mitochondrial analyses suggest that swordfish can be divided into four distinct stocks: Mediterranean, North Atlantic, South Atlantic, and Indo-Pacific (Alvarado Bremer et al. 1996; 2005; Chow et al. 1997; Rosel and Block 1996). While the structure of these stocks has been confirmed using nuclear genes, the boundaries between stocks have not been well defined (Chow and Takeyama 2000; Greig et al. 2000). A clear understanding of stock structure is required in order to develop more effective fishing management strategies (Ward 2000).

Microsatellite markers have previously been isolated and characterised from swordfish (Kasapidis et al. 2009; Reeb et al. 2003). These markers have consisted of dinucleotide, trinucleotide or complex repeats. Studies have typically used a small number of microsatellite markers in their population studies, ranging from four to six loci (Jean et al. 2006; Kasapidis et al. 2007; Kotoulas et al. 2007; Ward et al. 2001). It has only been in recent years that the number of loci has risen above ten (Kasapidis et al. 2008; Muths et al. 2009). The number of alleles reported per locus ranges from four to 82.

Total genomic DNA from a single swordfish was isolated using Qiagen DNeasy spin columns. Approximately 100µg was sent to Genetic Identification Services (California), where the DNA was enriched and screened for four tetra repeat motifs (CAGA, CATC, TAGA, and TGAC). Tetra-repeats were chosen in order to reduce the presence of stutter bands and increase the chance of accurate scoring. Imperfect repeat motifs were excluded to reduce difficulties in recognising clear alleles.

Primers were designed in the flanking regions of 29 clones using OLIGO v 6 (Natural Biosciences Inc.). Each primer set consists of a primer with a short A-tail sequence to promote adenylation of PCR products (Brownstein et al. 1996), and one primer with a sequence tag that is recognised by a third, fluorescently labelled primer (FAM, VIC, PET or NED). The sequence tag chosen was a CAG tag (Schable et al. 2002), with the sequence: 5' – CAGTCGGGCGTCATCA – 3'.

The 29 primer sets were initially tested on 16 swordfish samples. Primer sets were discarded if they failed to amplify, amplified multiple fragments or were difficult to amplify. The remaining primer sets were subsequently genotyped for 94 samples collected from eastern (Coral Sea, *n*=55) and western (Fremantle, *n*=39) Australia.

PCR amplifications were performed in 20µL reactions containing 40ng of template DNA, 1x reaction buffer, 1.5mM $MgCl_2$, 0.1mM dNTP's, 0.1µM fluorescent primer, 0.lµM a-tailed primer, 0.01µM tagged primer, and 1U Taq polymerase (FastStart, Roche). The PCR cycling consisted of an initial denaturation at 95^{o}C for 12 minutes, followed by 30-35 cycles of denaturation at 95^{o}C for 30 seconds, annealing at the appropriate temperature (Table 1) for 30 seconds, and extension at 72^{o}C for 1 minute. This was then followed by a final extension at 72^{o}C for 12 minutes.

Fragments were separated by the Australian Genome Research Facility (AGRF), using an (AB)® 3730 DNA Analyzer, incorporating a LIZ 500 size standard. Alleles were scored using GeneMapper® v 4.0 (Applied Biosystems). GenAlEx v 6.2 (Peakall and Smouse 2006) was then used to calculate expected and observed heterozygosities (H_E and H_O), as well as to test Hardy-Weinberg equilibrium (HWE).

Of the 29 primer sets tested on 16 samples, two failed to amplify, one amplified multiple fragments and an additional four amplified inconsistently and resulted in ambiguous genotypic data. The remaining 22 primer sets were then further optimised for 94 samples (Table 1).

The allele count for the 22 loci ranged from 2 to 26, with an average of 9.8. Two of the markers showed little variation; SwoB8 only had two alleles whereas the majority of samples were monomorphic for SwoB103 (allele frequency=0.956). SwoB124 proved difficult to score, with samples often containing more than two peaks. These three markers are likely to be of limited use in assessing population structure.

The SwoD2 clone sequence contained two microsatellite repeats, a tri- and a tetranucleotide repeat, separated by approximately 120 base pairs. Primers were designed for each repeat motif in order to compare the performance of the two microsatellites. Of the two markers, SwoD2B was easier to score and showed greater variation than SwoD2A.

Observed and expected heterozygosities (H_O and H_E) as well as Hardy-Weinberg equilibrium (HWE) are shown in Table 2. Five of the 22 loci failed to meet HWE. All deviations from HWE were due to heterozygote deficiency, with the exception of SwoA115. Only one locus, SwoB9, failed to meet HWE for both populations. These deficiencies could be due to null alleles. It is also possible that the observation of reduced heterozygosity could be attributed to sampling from regions where admixture of differentiated populations occur, known as the Wahlund effect.

The effective management of swordfish requires an understanding of the population structure of the species, particularly the structure within the four major stocks. This article has presented 22 microsatellite markers, 18 of which are likely to be of use in assessing population structure of swordfish.

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Tables

Table 1. Characteristics of 22 microsatellites loci for swordfish

Locus	Repeat Motif	Primer Sequence	Ta	Allele Range
SwoA3	(GACA)6	F: CAGTCGGGCGTCATCAAGTGAACCATCAGCGGCTCCT	58	95-115
		R: GTTTCATCCTTGACTGGCACCTCCG		
SwoA4	(GACA)6	F: CAGTCGGGCGTCATCAGGGCAAGTAGATAACAGAATTA	60	264-300
		R: GTTTCTTAGCCCATCACCCAATCCATCGT		
SwoA7	(GACA)6	F: CAGTCGGGCGTCATCAAGCAGACTCTGAGCCAAGTGCAA	62	271-283
		R: GTTTCTTCATCACCAATCAGCCACC		
SwoA8	(GTCT)7	F: GTTTCTTGCCCTTGCCTGGAG	58	222-238
		R: CAGTCGGGCGTCATCAGTGTTGGCAGGTGGTCTGGAG		
SwoA10	(CAGA)10	F: CAGTCGGGCGTCATCAGATTAAGGCAGCGGAGTCGAG	58	349-369
		R: GTTTCTTCGCTGGCAAGGCATTAGTTCAG		
SwoA113	(TCTG)6	F: GTTTCTTTCGCTGACAGACTTTACGACA	54	212-226
		R: CAGTCGGGCGTCATCAATCAGCTTCCAGGACAACACA		
SwoA115	(ACAG)8	F: CAGTCGGGCGTCATCAGCAAATGTGTTTAGCCGGAGA	58	379-495
		R: GTTTCTTTCCTGAATGGCAGTAATTGTG		
SwoB4	(GGAT)6	F: CAGTCGGGCGTCATCACCAATGCTCCTTTGTGTCGAA	58	228-256
		R: GTTTCTGCCATCACTGGAAAAAGTATCAGAGGTATG		
SwoB6	(GGAT)6	F: GTTTCTTGTGTACAGGATAACCGTCTTT	52	240-258
		R: CAGTCGGGCGTCATCAAGGGCAGTCAATTAGGTAGGC		
SwoB8	(GGAT)5	F: CAGTCGGGCGTCATCACTCAAGGATAAGAGCACTAGC	58	128-132
		R: GTTTCGATGAATGAACGGAAGTTTATCGGACTGTC		
SwoB9	(TCCA)8	F: GTTTCTTCGCTTTTGGTAGGGTACAGT	51	315-353
		R: CAGTCGGGCGTCATCATGTTATTCATCAGCGAGTGA		
SwoB103	(GGAT)3	F: GTTTCTTACACTTTGTTGTGCGGACTGG	58	214-222
		R: CAGTCGGGCGTCATCAGAAATCACATCAGCGTTACAC		
SwoB108	(CCAT)13	F: CAGTCGGGCGTCATCATTCAGTTTGTTGGCAGTTATT	58	166-226

		R: GTTTCTTCCATCCAGCCCTCCACTTATT		
SwoB112	(GATG)15	F: CAGTCGGGCGTCATCAGTTTATGTCAGCACAAGCACC	50	206-254
		R: GTTTCTTCTGCAAGTTTCACCGTTTCTA		
SwoB124	(GATG)11	F: CAGTCGGGCGTCATCATTGCTCCTTTAAGTATATCCA	56	321-363
		R: GTTTCTTAATGTAGCAAGTCGGCACACT		
SwoC4	(TAGA)12	F: GTTTCTTATCCGTCTCAGAGCAACTGGC	56	448-516
		R: CAGTCGGGCGTCATCACTCTTAGTGACCCACGGGAAT		
SwoC7	(GATA)18	F: GTTTCGGAACGCACATGCAGAGCTTA	56	216-288
		R: CAGTCGGGCGTCATCATTGGTCAAAGCTGCTCATATC		
SwoC8	(CTAT)22	F: CAGTCGGGCGTCATCACCTTCAATGTAGAGATGGCAGG	58	152-236
		R: GTTTCAAATGTCGGTGGAGCTGTGGACAGA		
SwoC10	(CATA)14	F: CAGTCGGGCGTCATCAAATGGAGACTGCGATTAAGAT	52	194-270
		R: GTTTCTTCAGTCTTTCTGCCATAACTCA		
SwoD2A	(TCC)7	F: CAGTCGGGCGTCATCACTCAAACTGAGACTTTCCAAGTAATCCT	58	282-297
		R: GTTTCACTTCCAGCCAAACTCTTGTTCGT		
SwoD2B	(CAGT)8	F: CAGTCGGGCGTCATCAAAGCAACAACATTGTCTTCTG	58	157-185
		R: GTTTCTGGCGTGAACGTGGCTCAATCC		
SwoD11	(TCAG)7	F: AGTCGGGCGTCATCAATGCAGGATTCCGCTGACCAGT	54	233-245
		R: GTTTCTTTGGATGTGGATATACGGCACC		

Table 2. Summary statistics for 22 microsatellite loci for swordfish
Number of alleles (N_a), expected (H_E) and observed (H_O) heterozygosities, and Hardy-Weingberg equilibrium (HWE)

		Coral Sea	*n*=55		Fremantle	*n*=39		Total		
Locus	**N_a**	**H_E**	**H_O**	**HWE**	**H_E**	**H_O**	**HWE**	**H_E**	**H_O**	**HWE**
SwoA3	6	0.666	0.500	0.325	0.676	0.526	0.497	0.671	0.513	0.346
SwoA4	8	0.470	0.500	0.707	0.405	0.419	0.945	0.438	0.453	0.836
SwoA7	5	0.356	0.291	0.086	0.494	0.564	0.956	0.425	0.428	0.578
SwoA8	6	0.107	0.093	0.000	0.234	0.256	1.000	0.170	0.175	0.987
SwoA10	6	0.689	0.673	0.835	0.654	0.718	0.835	0.671	0.695	0.786
SwoA113	6	0.649	0.679	0.932	0.584	0.649	0.962	0.621	0.665	0.609
SwoA115	26	0.898	0.816	1.000	0.857	0.906	0.040	0.879	0.863	1.000
SwoB4	8	0.139	0.145	1.000	0.257	0.256	0.804	0.187	0.188	0.709
SwoB6	5	0.430	0.241	0.008	0.464	0.270	0.047	0.467	0.272	0.003
SwoB8	2	0.245	0.245	1.000	0.29	0.245	0.330	0.265	0.244	0.473
SwoB9	10	0.704	0.698	0.108	0.718	0.553	0.000	0.715	0.619	0.000
SwoB103	3	0.108	0.075	0.630	0.051	0.053	0.868	0.084	0.066	0.110
SwoB108	10	0.757	0.646	0.000	0.787	0.611	0.125	0.772	0.632	0.000
SwoB112	12	0.852	0.808	0.018	0.860	0.868	0.301	0.857	0.838	0.000
SwoB124	15	0.759	0.932	0.612	0.753	0.750	0.995	0.769	0.850	1.000
SwoC4	17	0.885	0.800	0.060	0.882	0.806	0.004	0.892	0.855	0.000
SwoC7	15	0.910	0.884	0.941	0.891	0.917	0.623	0.899	0.906	0.831
SwoC8	21	0.912	0.977	0.318	0.926	0.865	0.848	0.919	0.923	0.902
SwoC10	18	0.913	0.922	0.552	0.926	0.838	0.423	0.920	0.880	0.616
SwoD2A	4	0.469	0.509	0.794	0.431	0.405	0.907	0.454	0.467	0.883
SwoD2B	8	0.656	0.759	0.721	0.673	0.703	0.833	0.665	0.733	0.958
SwoD11	5	0.614	0.691	0.882	0.605	0.718	0.731	0.610	0.704	0.823