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TECHNICAL NOTE

Isolation and characterization of 12 microsatellites for the commercially important sablefish, *Anoplopoma fimbria*

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Abstract Sablefish, *Anoplopoma fimbria*, are long-lived, highly migratory, bathydemersal, commercially important fishes that inhabit continental slope waters of the North Pacific Ocean. Here we describe 12 microsatellite loci developed for sablefish, and cross-species amplification in skilfish, *Erilepis zonifer*. Microsatellites were developed from one sablefish and characterized using 55 juveniles collected in the eastern Gulf of Alaska. The number of alleles ranged from 3 to 27 per locus, and observed heterozygosity ranged from 0.074 to 0.964. There was no significant evidence for linkage disequilibrium or departure from Hardy–Weinberg Equilibrium. Ten of the 12 microsatellite loci were successfully amplified in skilfish. These new microsatellites were developed for use in sablefish fishery management and conservation applications including selecting broodstock for aquaculture operations and defining population boundaries for stock assessments.

Keywords Alaska · *Anoplopoma fimbria* · *Erilepis zonifer* · Microsatellites · Sablefish · Skilfish

Defining genetic stock structure of commercially important marine species is essential for sustainable fisheries management and conservation. Sablefish, *Anoplopoma fimbria*, are large, long-lived, highly migratory, bathydemersal fishes that inhabit continental slope waters of the North Pacific Ocean. Sablefish are a valuable resource and are thought to form northern and southern populations based upon tagging data, size at maturity, and growth rate patterns (Kimura et al. 1998). Despite these phenotypic differences, early genetic studies using allozyme loci did not detect regional genetic stock structure throughout the species range (Gharrett et al. 1982). However, modern DNA-based markers, such as microsatellites, may provide increased power to detect subtle population structure (Shaw et al. 1999). Here we report on the isolation and characterization of polymorphic microsatellites for sablefish, and cross-species amplification in skilfish, *Erilepis zonifer*, the only other member of the family Anoplopomatidae.

Fifty-five juvenile sablefish were collected in the eastern Gulf of Alaska aboard the research vessel (R/V) *Medeia* from 12 to 14 August 2005 (Orsi et al. 2006), and one skilfish was collected aboard the fishing vessel (F/V) *Ocean Prowler* while sablefish fishing, 25 July 2011. Genomic DNA from all 56 specimens and one additional sablefish collected by the NOAA ship *Oscar Dyson* was extracted with a Qiagen® DNeasy® Blood & Tissue Kit.[1] A microsatellite enriched library was constructed with the NOAA-obtained sablefish following the methods of Glenn and Schable (2005), with the following modifications: 5 μg of total DNA was digested with *Rsa*I to fragment the DNA to approximately 500 base pairs (bp). Enriched microsatellite DNA was amplified by PCR using the Advantage 2 polymerase (Clonetech®) with an extension temperature of 68°C. Amplification products were cloned into a high-copy T/A plasmid-based cloning vector pGEM® T-easy and electroporated into *Escherichia coli* DH10b electrocompetent host cells (Invitrogen™).

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[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Transformants were selected on LB plates containing 300 μg/mL carbenicillin. Recombinant colonies were picked robotically using a QBot (Genetix) and stored as individual clones as glycerol stocks at –80°C. Plasmid DNA was purified using standard alkaline lysis conditions (Sambrook et al. 1989) and 192 individual clones were sequenced using BigDye® chemistry [Applied Biosystems (AB)]. Microsatellite repeat arrays were identified with MSATCOMMANDER software (Faircloth 2008) and primers were initially designed with M13 tails (Schuelke 2000) using PRIMER3 software (Rozen and Skaletsky 2000).

Primers were identified in 63 of the 192 microsatellite-enriched clones, and loci were screened for variability in 8 sablefish using universal M13-tagged fluorescent labeled primers (Schuelke 2000). 10 μl PCR reactions were performed in a GeneAmp® PCR System 9700 (AB) with GoTaq® Flexi DNA Polymerase (Promega), 10 mM dNTP mix (Fermentas), 1 μM M13 tagged primer, 5 μM untagged primer, and 5 μM 6FAM labeled M13 tag, using a thermal cycling routine of 5 min at 94°C, 30 cycles of 30 s at 94°C, 45 s at 56°C and 45 s at 72°C, 8 cycles of 30 s at 94°C, 45 s at 53°C, and 45 s at 72°C, followed by a final extension of 10 min at 72°C. Fragments were analyzed on an ABI 3130xl (AB), and alleles were read using GeneMapper® (AB).

Twelve of the 63 primer pairs were variable and produced readable peaks. Genetic variation at 12 microsatellite loci was assayed on 55 sablefish samples and one skilfish sample using a Qiagen® Multiplex PCR Kit following the manufacturer's protocols. Loci were separated by fluorescent dyes (6FAM, VIC, NED, or PET) using the equipment described above. Exact Hardy–Weinberg Equilibrium (HWE) probability tests (Guo and Thompson 1992) and log-likelihood *G* tests for genotypic linkage disequilibrium were conducted with 1,000 batches of 1,000 Markov Chain (MC) iterations following 1,000 MC iterations of burn-in, and gene diversities were calculated following Weir and Cockerham (1984). All statistical analyses were conducted in GENEPOP 4.0.10 (Raymond and Rousset 1995), and *P*-values were corrected for

Table 1 Characteristics of 12 sablefish (*Anoplopoma fimbria*) microsatellites, including GenBank accession, annealing temperatures (T_A), samples sizes (*n*), numbers of alleles (*k*), observed and expected heterozygosities (H_O and H_E, respectively), and exact *P*-values of Hardy–Weinberg Equilibrium (HWE) probability tests

Locus	GenBank accession	Primer sequences (5′–3′)	T_A (°C)	Repeat motif	*n*	*k*	Allele size (bp)	H_O	H_E	HWE *P*
AFI1*	JN872646	TGAAATGGCCTTGAGCTGG GGGGCTGAACTCTCCTCTG	63	$(AG)_8$	55	3	161–165	0.491	0.523	0.774
AFI2*	JN872647	TTTGGATGACGTGTGTGCC TGAGGCTGAGCAGCAATGG	63	$(CT)_6(ATCT)_4$	54	17	157–202	0.944	0.887	0.598
AFI3*	JN872648	TGGTCCCAGTAGTCTGTTGC GGAACTGTCACCCTGAATGC	64	$(AG)_6$	55	4	166–174	0.473	0.507	0.272
AFI4	JN872649	GCCTGTAAATTTCCCCGCTG TCACCAACAGCTCCCTCAC	64	$(ATCTT)_5$	54	3	175–185	0.352	0.332	0.848
AFI5*	JN872650	GTTCATCCTGATATGCGGGG AGCTGAGGTGATGCTGAGG	64	$(GT)_8$	55	26	189–290	0.927	0.916	0.265
AFI6*	JN872651	CGCCACAGAAATCGAACCG GCTCTTGCTGAGATGTGCG	65	$(AC)_9…(AGGG)_3$	53	15	182–256	0.849	0.851	0.064
AFI7*	JN872652	ACCCCTAAGGAACGCTTGG AAACAGGAACCGGTCTGGG	65	$(CT)_{12}$	53	10	181–210	0.642	0.693	0.538
AFI8	JN872653	CATTGGAGCTTGCAGGCAG CTGTCTCGCGAAAAGTCTGG	64	$(AC)_7…(AC)_7…(AC)_{18}$	53	20	185–256	0.943	0.899	0.833
AFI9*	JN872654	AGCGTGAGATGCTGATTGG CGCTGCTCTTTCTCTCTGC	63	$(CT)_8…(AGGG)_3$	50	11	212–223	0.820	0.807	0.542
AFI10*	JN872655	TCTTTGCTGCTGCATCGTG CGGCTCTTGCCAATGCTTC	63	$(AG)_8…(AG)_8$	52	10	195–214	0.808	0.842	0.085
AFI11*	JN872656	TCACTCCTACTGGTCTAGGG ACAGGGCATTGAAATGACAGC	62	$(ATCT)_{32}…(ATCT)_3$	55	27	108–255	0.964	0.955	0.851
AFI12*	JN872657	TCACACTAATCCTTTGTGAGTGAG TCTTTCAGTGCCGCTCTTTAC	62	$(AG)_5…(GT)_6$	54	3	212–222	0.074	0.090	0.038

* Amplified in skilfish, *Erilepis zonifer*

multiple comparisons by dividing the 5% cutoff significance level by the number of loci tested, for corrected table-wide significance at $P \leq 0.004$.

Of the 55 sablefish assayed at 12 loci, complete multilocus genotypes were recovered from 43 individuals, 7 individuals were missing a single genotype, and 5 individuals were missing two genotypes. One of the 66 log-likelihood G tests for genotypic linkage disequilibrium was significant (AFI1 and AFI5; $P = 0.043$), and there was significant departure from HWE at AFI12 ($P = 0.038$; Table 1), but no tests remained significant after correction for multiple comparisons. Average observed heterozygosity was 0.691 and ranged from 0.074 in AFI12 to 0.964 in AFI11. Average expected heterozygosity was 0.692 and ranged from 0.090 in AFI12 to 0.955 in AFI11 (Table 1). The average number of alleles was 12.4 and ranged from 3 in AFI1, AFI4, and AFI12 to 27 in AFI11. Ten microsatellites were successfully amplified in skilfish (Table 1).

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