TECHNICAL NOTE

Development of fifty-one novel EST-SSR loci for use in rockfish (genus *Sebastes*)

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Abstract We mined expressed sequence tag libraries from three species of rockfish (*Sebastes caurinus*, *S. goodei*, and *S. rastrelliger*) for microsatellites. A total of 111 novel sequences with repetitive elements were isolated and we were able to successfully amplify 51 of these in five different rockfish species. Population level surveys for two species (*S. flavidus* and *S. melanops*) were conducted with these 51 loci. Twelve loci showed deviations from Hardy–Weinberg equilibrium, indicating the presence of null alleles or duplicated loci. Eleven of the 51 loci annotated to the SWISSPROT database. This suite of loci will prove useful in current and ongoing conservation and genomic studies of rockfish.

Keywords *Sebastes* · EST-SSR · Genomics · Stock identification

The genus *Sebastes* is a large group of saltwater fishes (>100 species currently recognized) that inhabit a diverse array of ecological niches in the marine environment (Love et al. 2002). Rockfish have been intensively studied from a genetics perspective (Burford and Bernardi 2008; Buonaccorsi et al. 2004; Gilbert-Horvath et al. 2006; and others) as they are part of many commercial and recreational fisheries and serve as useful models for studying marine systems. Here we describe a set of expressed sequence tag (EST)-microsatellites that can be used to explore ecological/evolutionary questions and supplement the conservation and management efforts for this group of fishes.

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We used three independent EST datasets for microsatellite discovery. The first two sets of EST sequences were downloaded from GENBANK for *S. rastrelliger* and *S. caurinus* (accession numbers: GE796994-GE820661 and EW975926-EW987132). Given the close affinity of *S. caurinus* and *S. rastrelliger* (Hyde and Vetter 2007), we assembled ESTs from both species with CAP3 (Huang and Madan 1999) using default parameters. The third set of ESTs used for microsatellite discovery was developed from *S. goodei* ESTs (ovary and testes tissue—Aguilar et al. in prep). All *S. goodei* ESTs mined for microsatellites were previously checked for quality, trimmed and assembled with CAP3 (Aguilar et al. in prep). Microsatellite searches were preformed with TANDEM REPEATS FINDER v4.04 (Benson 1999) and primers were designed with PRIMER3 v0.4.0 (Rozen and Skaletsky 2000).

Microsatellites were amplified using a three-primer amplification which includes a fluorescently-labeled M13 primer (Schuelke 2000). Reactions were run in 15 µl volumes with the following conditions: 1X ABI buffer, 2.5 mM $MgCl_2$, 0.4 mM dNTPs, 2.7×10^{-4} mg/ml BSA, 0.3 µM reverse primer, 0.3 µM fluorescently-labeled M13 sequence (5′-CACGACGTTGTAAAACGAC-3′) using Applied Biosystems Inc. (ABI) dye labels (FAM, VIC, NED, PET), 0.07 µM M13 5′-end labeled forward primer, 0.2 units of ABI Taq polymerase, and 5 µl of DNA (5–20 ng/µl). We used the following thermal profile: 95°C for 3 min, followed by 10 cycles of 94° for 30 s, 58°C for 45 s ('touching down' 1°C each cycle), 72°C for 45 s. This was followed by 35 cycles at 94°C for 30 s, 48°C for 30 s, and a 72°C for 45 s, and a final extension of 72°C for 7 min. Hundred and eleven novel primer pairs were tested on three rockfish species: *S. goodei* ($N = 3$), *S. flavidus* ($N = 3$), and *S. melanops* ($N = 2$). Products were run on an agarose gel for confirmation of amplification and those

Table 1 Primers for 51 polymorphic EST-derived microsatellites

Locus/Genbank Acc. no.	Primer set (5′–3′)	Motif	Range	Annotation
Sras112/EW985432.1	F-CGGCCATCTGGTTCTAATC	$(TG)_{22}TC(TG)_3$	152–196	–
	R-CGTGGTCTTAAACACATTGC			
Scau306/GE806111.1	F-TCTCTTTCCCTTCCCTCAAT	$(AC)_3AA(AC)_{11}TA(AC)_{10}$	164–176	s-phase kinase-associated protein 1
	R-CCATGAAACCGGGTCTATTT			
Sras320/EW983759.1	F-GCCACTTATTTCAGGCATGT	$(AG)_{12}$	111–121	–
	R-CATGGACACAATGAGGGTCT			
Sras345/EW984164.1	F-GCCACTTATTTCAGGCATGT	$(TC)_{12}$	111–121	–
	R-CATGGACACAATGAGGGTCT			
Sras413/EW984981.1	F-GAGTGGTGGTGGGGTTTAAT	$(AC)_7GA(AC)_3TA(AC)_5$	170–182	–
	R-TACCTGCGCAGTAAATGCTT			
Scau506/GE804468.1	F-AGCCAGATCTGATGATGTCC	$(TC)_{11}$	151–155	–
	R-AGCTTGAGAGTGCGACAGAT			
Sras1856/EW982965.1	F-CAAGCTGCACCTTTAACCAC	$(AAG)_{15}$	106–124	–
	R-GCCCTTAAAGAGCATTAGGC			
Sras309791/EW983522.1	F-CCGGCAAGATGATTAGTGAG	$(AC)_{14}$	138–138	–
	R-TTGGACTTCAATTGGTGGAT			
Sras309817/EW983581.1	F-CCTGGACGTCAACCTTTTTA	$(AC)_{18}$	103–115	–
	R-CACCGACTACAAAATGCACA			
Sras310745/EW985747.1	F-GCAAGTTTTGAGCTTTGGTT	$(TGTT)_7$	127–151	–
	R-CTACATGGCAATCCCTACCA			
Sras311101/EW986599.1	F-GAGAATGTTTGGGACAATGC	$(CA)_{13}$	141–145	
	R-CCAGCGTTCAGTCAGAAGAC			
Sras311217/EW986866.1	F-ACTCAGCTGTCTGCATCTCA	$(CA)_2NN(CA)_{11}NN(CA)_4$	161–165	–
	R-GCAGCATGCATCCTTGTAT			
Sras311560/EW979523.1	F-TGTGGGTATGCGTCAGTATG	$(TG)_{14}$	142–148	–
	R-CATGCGTCATTCAAAACTCA			
Sras311860/GE807252.1	F-ACGGAGGGGAACATAAAGG	$(CA)_{12}$	169–177	Growth hormone-releasing factor
	R-CAGGGAGGATGTAGGATGTG			
Sras311899/EW980302.1	F-CGATCCATGAGTCAATCTCAC	$(AC)_{18}$	160–170	–
	R-GGTTGGTGTGTGAATGTGTG			
Sras312105/EW980788.1	F-GAAGGATTTACGACATGACCA	$(TTC)_{10}$	133–148	Zinc finger five domain-containing protein 16
	R-CATTTCGGTAGAGCGTCTGT			
Sras312558/EW980263.1	F-ATTTTCATCCTGTTTGTCCA	$(TG)_{13}$	117–119	–
	R-TTAAAGCCAGTCCCTGAAAA			
Scau356962/GE799327.1	F-CGTGTGTTTTATATGTGGCATT	$(TG)_{21}$	174–192	–
	R-AAACGCCAACTTCATGACTG			
Scau357863/GE800773.1	F-ACTAATGCATGAAGGCCACT	$(TTC)_{18}$	90–135	–
	R-ATCAGTAGAGGGCAGCAATG			
Scau359573/GE803879.1	F-TGCCTATACAACCTGCTGCT	$(TCTA)_{10}$	122–154	–
	R-TCATATCGCCAAATTCTTGTT			
Scau361657/GE802559.1	F-AAGGTGTGACGACGACTACAA	$(TG)_{12}$	127–127	–
	R-CGGATGTGCCTCTCATAAAC			
Sgoo3582a/HS573393	F-GAAGATGGTTTGTGCCACATT	$(ACAG)_{28}$	127–139	–
	R-CCCTGTGATCTGTCTGTCTGTC			
Sgoo1801a/HS573394	F-CAGGGACTTGGTGTCACAGTT	$(AGAT)_{24}$	177–241	
	R-CTTTTCCTGTGCATTCTTGGA			
Sgoo1193_3/HS573418	F-CAGTTCAAACATGCCACAACA	$(AC)_{20}$	169–173	–
	R-CCTCTGTAAGCAACCATCAGC			

Table 1 continued

Locus/Genbank Acc. no.	Primer set (5′–3′)	Motif	Range	Annotation
Sgoo690_1/HS573395	F-TGAAACAACAACACACTCCTGA R-CTCTAAAGGACTCCCCTGTGC	$(AC)_{23}$	68–108	–
Sgoo930_1/HS573419	F-GGCTGTGTGATCGTATCTGCT R-CTCCTTTATGGTGGATGCTGA	$(ACTG)_{10}$	106–178	Synaptobrevin homolog YKT6
Sgoo5909_1/HS573396	F-CCGCACATGTTACAACAACAA R-CTCATCCTCACCATGGCTTG	$(AGC)_{11}$	109–125	–
Sgoo3574_1/HS573397	F-GATTCAGTTGAGAACAAAGAGC R-CTTGATTAAGCCCCAATGTT	$(AC)_{17}$	115–131	–
Sgoo5968_1/HS573398	F-TCAACACCCTATTGAAGCACA R-ACTCCTTTGCATTAGGGGAGA	$(AGC)_{10}$	114–117	Protein Tob1
Sgoo10_911_1/HS573399	F-GTTTACGTGCTGTGCTGCTTT R-AATCTGCTGATTTAGGGGAATC	$(AAAG)_{9}$	107–107	Protein LBH
Sgoo1190_1/HS573420	F-CGTCCAGAGTGAAGAGTTGCT R-TGAAGCAGACATGAACCAGAA	$(ATC)_{9}$	127–235	Src substrate cortactin
Sgoo3931_1/HS573400	F-GACTGTTTCTGGATGTGAGACTG R-GCTGAGTTGGACCCTTCTCTC	$(AC)_{13}$	133–147	–
Sgoo6768_1/HS573401	F-GGGTTCCTTGTTCTCTTGGTT R-TTCTCGTGAAGGTTCTGGTTC	$(AAT)_{9}$	114–135	–
Sgoo642_1/HS573402	F-ATCTTTGCCAAGGCCGTAAT R-GGAGGTTATGTTTTCGGTTCG	$(AAT)_{9}$	172–187	–
Sgoo2027_1/HS573403	F-CAGACTTTCAGCCACTTTTGG R-ACACCTGCTGTCAAATCTGCT	$(AAAG)_{7}$	133–145	Protein LBH
Sgoo5877_1/HS573404	F-CGGCCAAACCCATATTCTTAT R-GGCGGGGACAGTATTCAGTTA	$(AAT)_{9}$	103–105	–
Sgoo583_1/HS573421	F-CAGGAACAGGTTAAACCAGCA R-AGTAAAGAATGGCGTCCAGGT	$(AG)_{13}$	93–109	–
Sgoo2408_1/HS573405	F-GCAGCAAAGGGAAGAAAAGAG R-GCTGTAGAGTCAAACCGCAGA	$(AG)_{13}$	167–209	Acyl-CoA desaturase
Sgoo7119_1/HS573406	F-GCTGACTGTGTGCACCAACTA R-CAAGCTGGCACCTTACTTGAG	$(AC)_{13}$	167–171	Synaptotagmin-like protein 1
Sgoo4874_1/HS573407	F-GCAACCAACAGAAGAACAAGC R-GCTGTGGGTTCCGTTCTTATT	$(ATC)_{9}$	149–164	–
Sgoo4_308_1/HS573408	F-CATGGCGTACAACCAGGAATA R-GGGGTTTCTCTCCATCAGAAG	$(AC)_{16}$	175–185	–
Sgoo9_832_1/HS573409	F-AAGACCTGAAGGAGCTCAAGG R-GTGGTTGAGGGTGTTTCAGAC	$(AGG)_{11}$	125–138	Semaphorin-3F
Sgoo1765_1/HS573410	F-TGCTTAATACCATGGGACAGG R-TCACAACACCAATGCAGACTT	$(AAT)_{11}$	137–137	–
Sgoo8_703_1a/HS573411	F-GTGAAGCCCTCAAACTTTCAA R-CTGTACAAGTAGCATCGGATTGTT	$(AAT)_{13}$	79–85	–
Sgoo2916_1a/HS573412	F-CTCTTGGCTGTTTTGTTGCTC R-CTCTCTCGTGCTTCACCACTC	$(AC)_{12}$	124–127	–
Sgoo1842_1a/HS573413	F-AGGAGAGAGCCAGGGATCTAA R-GAACACTGCCCATGATGTTG	$(AC)_{12}$	139–161	–
Sgoo742_1/HS573422	F-GCTGACACAAGTTTGTATGTGTTG R-CTTTTCTGTGTCAAGGCCACT	$(AC)_{15}$	157–171	–
Sgoo3644_1/HS573414	F-TTCGCTGTGCAGTCAACTAGA R-GACCTCAGGAGTGGTGATTGA	$(AC)_{15}$	135–139	–

Table 1 continued

Locus/Genbank Acc. no.	Primer set (5′–3′)	Motif	Range	Annotation
Sgoo4_310_1/HS573415	F-GACACTCCAGGACAGAGGACA	$(AC)_{11}$	148–160	–
	R-TGACTGCTAGAGGAAGCAGGA			
Sgoo3699_1/HS573416	F-CTACATAGAGCAGCTTCATCGTC	$(AT)_{10}$	105–107	–
	R-CGTTGTTTTGTCCACCGTTAT			
Sgoo813_1/HS573417	F-TTACAGCTCCAGTGGCTTCTC	$(AC)_{18}$	118–120	–
	R-GCTACATGCACCCATGGTATT			

Genbank accession numbers, repeat motifs, observed allele size ranges and annotations to the SWISSPROT database are also reported

Table 2 Sample size (N), observed number of alleles (k), observed (H_o) and expected (H_e) heterozygosity for two populations of S. flavidus and S. melanops

Locus	S. flavidus—North				S. flavidus—South				S. melanops—North				S. melanops—South			
	N	k	H_o	H_e	N	k	H_o	H_e	N	k	H_o	H_e	N	k	H_o	H_e
Scau306	23	6	0.565	0.637	24	4	0.542	0.568	23	5	0.609	0.58	24	7	0.708	0.683
Scau356962	24	3	0.208	0.294	22	3	0.409	0.413	23	2	0.522	0.502	23	2	0.348	0.502
Scau357863	20	4	0.150*	0.317*	23	6	0.334*	0.552*	23	5	0.478	0.454	21	5	0.667	0.548
Scau359573	24	9	0.75	0.848	23	7	0.826	0.781	23	5	0.87	0.819	21	5	0.81	0.803
Scau361657	24	1	0	0	23	1	0	0	24	2	0.042	0.042	24	2	0.167	0.156
Sras112	24	11	0.875	0.748	24	12	0.917	0.854	23	2	0.087	0.085	24	3	0.167	0.159
Sras320	23	5	0.783	0.689	23	5	0.609	0.682	24	9	0.667	0.707	22	6	0.591	0.617
Sras345	24	5	0.792	0.697	24	6	0.625	0.688	24	9	0.667	0.707	24	6	0.583	0.638
Sras413	23	4	0.652	0.596	24	6	0.667	0.606	24	4	0.417	0.48	24	5	0.583	0.567
Sras506	21	2	0.524	0.512	19	3	0.526	0.522	24	2	0.167	0.284	24	2	0.25	0.383
Sras1856	24	6	0.708	0.745	24	5	0.958	0.772	22	8	0.682	0.79	22	5	0.682	0.714
Sras309791	20	1	0	0	23	1	0	0	23	4	0.522	0.637	24	3	0.25	0.26
Sras309817	23	4	0.304	0.278	23	3	0.174	0.166	24	7	0.708	0.661	24	5	0.667	0.626
Sras310745	23	7	0.739	0.608	24	9	0.708	0.657	23	5	0.565	0.542	22	5	0.727	0.623
Sras311101	21	3	0.143	0.138	22	3	0.227	0.21	23	3	0.652	0.498	23	4	0.652	0.559
Sras311217	22	2	0.091	0.089	23	3	0.565	0.492	23	2	0.13	0.125	17	1	0	0
Sras311560	23	3	0.435	0.426	23	2	0.522	0.487	19	4	0.368	0.368	23	2	0.087	0.085
Sras311860	23	3	0.478	0.474	24	4	0.417	0.444	24	3	0.25	0.231	23	3	0.174	0.166
Sras311899	23	3	0.522	0.463	22	2	0.318	0.333	22	4	0.5	0.576	15	5	0.533	0.676
Sras312105	24	3	0.125	0.121	24	4	0.208	0.197	23	4	0.304*	0.530*	24	4	0.542*	0.621*
Sras312558	24	1	0	0	24	2	0.125	0.12	23	2	0.217	0.198	24	3	0.167	0.159
Sgoo3582a	23	4	0.783	0.666	23	3	0.739	0.68	24	3	0.0833	0.082	22	3	0.091	0.09
Sgoo1801a	24	9	1	0.887	22	11	0.864	0.904	24	9	0.708	0.814	23	9	0.913	0.853
Sgoo1193_3	22	3	0.273	0.246	22	2	0.091	0.169	18	2	0.056	0.056	24	3	0.25	0.29
Sgoo690_1[a]	24	2	1*	0.511*	24	2	1*	0.511*	–	–	–	–	–	–	–	–
Sgoo930_1	24	12	0.875	0.905	24	12	0.833	0.871	–	–	–	–	–	–	–	–
Sgoo5909_1	23	5	0.609	0.515	23	2	0.609	0.407	24	2	0.458	0.403	22	3	0.318	0.369
Sgoo3574_1	23	4	0.217	0.206	23	6	0.435	0.415	22	7	0.636*	0.781*	21	8	0.714	0.711
Sgoo5968_1	21	2	0.048	0.048	21	1	0	0	24	1	0	0	24	1	0	0
Sgoo10_911_1	22	1	0	0	23	1	0	0	24	1	0	0	24	2	0.042	0.042
Sgoo1190_1	23	19	0.957	0.935	22	11	0.773	0.617	–	–	–	–	–	–	–	–
Sgoo3931_1	22	4	0.455	0.563	24	7	0.833	0.713	24	7	0.667	0.681	23	7	0.739	0.729
Sgoo6768_1	22	4	0.227*	0.325*	20	6	0.4*	0.7*	–	–	–	–	–	–	–	–
Sgoo642_1	23	3	0.478	0.498	22	4	0.5	0.536	19	2	0.263	0.235	24	2	0.125	0.12

Table 2 continued

Locus	S. flavidus—North				S. flavidus—South				S. melanops—North				S. melanops—South			
	N	k	H_o	H_e	N	k	H_o	H_e	N	k	H_o	H_e	N	k	H_o	H_e
Sgoo2027_1	20	1	0	0	23	2	0.043	0.043	24	1	0	0	24	3	0.042*	0.121*
Sgoo5877_1	23	2	0.087	0.085	23	2	0.043	0.043	24	2	0.083*	*0.422	24	3	0.042*	0.26*
Sgoo583_1	17	6	0.588*	0.77*	20	6	0.6	0.715	24	6	0.792	0.736	23	5	0.652	0.636
Sgoo2408_1	20	14	0.9	0.912	23	13	0.826	0.877	22	15	0.864	0.928	18	12	0.778	0.778
Sgoo7119_1	22	3	0.455	0.935	21	3	0.667	0.617	–	–	–	–	–	–	–	–
Sgoo4874_1	23	4	0.696	0.596	22	5	0.545	0.5	19	5	0.158*	0.509*	24	4	0.208	0.197
Sgoo4_308_1	24	4	0.25	0.233	24	4	0.375	0.438	23	6	0.696	0.703	23	6	0.783	0.696
Sgoo9_832_1[a]	23	2	1*	0.511*	21	2	1*	0.512*	–	–	–	–	–	–	–	–
Sgoo1765_1	24	1	0	0	23	1	0	0	24	3	0.125	0.121	24	1	0	0
Sgoo8_703_1a	21	2	0.143*	0.438*	24	3	0.083*	0.494*	–	–	–	–	–	–	–	–
Sgoo2916_1a	24	2	0.042	0.042	24	2	0.083	0.082	24	2	0.083	0.082	22	2	0.045	0.045
Sgoo1842_1a	16	6	0.625	0.673	24	7	0.625	0.627	–	–	–	–	–	–	–	–
Sgoo742_1	24	3	0.167	0.159	23	3	0.174	0.165	24	2	0.208*	0.403*	24	3	0.458	0.434
Sgoo3644_1	24	2	0.417	0.422	21	3	0.619	0.452	24	2	0.25	0.383	24	2	0.25	0.284
Sgoo4_310_1	21	6	0.571*	0.741*	21	5	0.333*	0.621*	24	3	0.292	0.37	24	3	0.458	0.393
Sgoo3699_1	24	2	0.042	0.042	23	2	0.261	0.232	22	2	0.045	0.045	22	3	0.273	0.246
Sgoo813_1	24	2	0.458	0.488	21	2	0.524	0.494	24	2	0.25	0.223	24	2	0.083	0.082

* Significant deviation from HWE ($P < 0.05$)

–, no data was obtainable for this locus (either no amplification or locus was unscorable)

[a] A possible duplicated locus

that displayed positive amplifications were run on an ABI3100 automated sequencer with LIZ-500 and genotyped using GENEMAPPER v4.0 (ABI). A total of 51 (out of 111) loci gave reliable and scorable products that we explored for within population variation (Table 1).

Forty eight S. flavidus and 48 S. melanops samples from two California (USA) populations (north and south of Cape Mendocino respectively) were used to assess population level variation using the aforementioned PCR protocol. Products were typed on an ABI3100 as above. Departures from Hardy–Weinberg equilibrium, allele counts (k), and levels of heterozygosity (H_{exp}, H_{obs}) were calculated using the program GENEPOP v4.0 (Rousset 2008). Loci that amplified and were scored successfully were annotated to the SWISSPROT database using the BLAST2GO software (Conesa et al. 2005). Data is only presented from loci that we were able to successfully amplify and genotype in S. flavidus and S. melanops. Primer information for loci that did not give successful amplifications, or were difficult to genotype, are available from the corresponding author.

After omitting fixed loci allele counts for S. flavidus and S. melanops ranges from 2 to 19 and 2 to 13, respectively. Observed heterozygosity ranged from 0.042 to 1.0 S. flavidus and 0.042 to 0.913 in S. melanops. Seven microsatellite loci did not conform to Hardy–Weinberg expectations ($P \leq 0.05$) in S. flavidus, while five loci did not conform to

Hardy–Weinberg expectations ($P \leq 0.05$) in S. melanops (Table 2). Two primer sets appear to be amplifying duplicated loci (Sgoo690_1 and Sgoo9_832_1) due to the fact all individuals that successfully amplified were heterozygotes (Table 2).

Eleven loci were annotated to the SWISSPROT database. The remaining 40 loci did not show any significant matches. Two of the loci (Sgoo10_911_1 and Sgoo2027_1_ annotated to the same protein (Protein LBH) but were not in linkage disequilibrium (data not shown). These EST-linked microsatellites with annotations may serve useful in the development of a linkage or genomics maps of the Sebastes genome and further assist in the management and conservation in this economically important group. Additionally, gene associated markers provide opportunities for evolutionary analysis of locally adapted genes in multiple Sebastes populations. This is the first set of EST-linked microsatellites in Sebastes and shows that there is significant cross-species amplification among other species in the genus.

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