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Microsatellite Markers for Red Drum, *Sciaenops ocellatus*

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Polymerase chain reaction (PCR) primers are reported for 68 nuclear-encoded microsatellites developed during the past several years from genomic libraries of red drum (*Sciaenops ocellatus*). All 68 microsatellites were tested for reproducibility and polymorphism on a sample of five to 12 red drum; 60 of the microsatellites were found to be polymorphic. Estimates of observed and expected heterozygosity (gene diversity) and tests of conformity of genotypes to Hardy–Weinberg equilibrium were carried out for a subset of 31 microsatellites on a larger sample of 45 adults provided by Texas Parks and Wildlife. Levels of allelic and gene diversity were average relative to values observed for marine and anadromous fishes. The set of genetic markers should be useful for a variety of studies, including monitoring and assessment of red drum stock enhancement.

The red drum, *Sciaenops ocellatus,* is an estuarine-dependent sciaenid fish found in the western Atlantic Ocean from Massachusetts to the Yucatan Peninsula, including the Gulf of Mexico (Pattillo et al., 1997). The species comprises an important recreational fishery in bays and estuaries of Gulf Coast states and along the Atlantic coast of the southeastern United States (Swingle, 1987; Van Voorhees et al., 1992). Because of significant declines in red drum abundance stemming from overfishing and habitat deterioration (Heffernan and Kemp, 1982; Swingle et al., 1984), recovery plans were implemented in nearly all Gulf Coast and Atlantic states. These recovery plans included assessment of red drum stock structure and in some states, principally Texas, stock enhancement with hatchery-raised fingerlings (McEachron et al., 1995).

Previous studies of red drum population genetics have used a variety of genetic markers and shown that 1) red drum in the Gulf comprise a different stock than red drum along the Atlantic coast (Bohlmeyer and Gold, 1991; Gold et al., 1993) and 2) population structure of red drum in the northern Gulf follows a modified one-dimensional, linear stepping-stone model (Gold et al., 2001). More recently, there has been an increasing interest for estimating the genetic component of traits important in red drum culture. This necessitates development of genetic markers that permit identification of kinship among offspring raised in the same environment from early life stages when physical tagging is impossible. For both types of studies, i.e., population structure and kinship analysis, deoxyribonucleic acid (DNA) microsatellites have proved to be a powerful tool. Briefly, microsatellites are short stretches of nuclear DNA composed of di-, tri-, and tetranucleotide arrays that are embedded in unique (specific) DNA flanking regions, inherited in a codominant fashion (Wright and Bentzen, 1994), and distributed throughout euchromatic regions of chromosomes (Weber and May, 1989; Weber, 1990). Variants at microsatellite loci are thought to arise rapidly (Schug et al., 1998), meaning that 1) recently diverged subpopulations (stocks) may be detected more easily with microsatellites than with other, commonly used genetic markers (e.g., mitochondrial DNA) and 2) the degree of genetic identity in microsatellite alleles may be used to estimate degree of genetic relatedness. When available in large number, microsatellites in principle can be used to carry out large-scale "family printing" for use in identifying hatchery-raised juveniles released into the wild, enabling evaluation of long-term survival and ecological performance of these fish. Large numbers of microsatellites also can be used to generate genetic maps and, ultimately, to localize quantitative trait loci or QTLs (Georges et al., 1995) of interest for both wild and cultured populations.

In this note, we report on polymerase chain reaction (PCR) primers and optimized annealing temperatures for the 68 microsatellites developed during the past several years in our laboratory from red drum genomic libraries. Most of the PCR primer pairs are published (Turner et al., 1998; O'Malley et al., 2003), but assays (primarily, annealing temperature) for many of the microsatellites were not optimized nor were data on gene diversity based on sample sizes of more than a few individuals. We also report data on observed and expected heterozygosity (gene diversity) and results of tests of conformity of genotypes to expectations of Hardy–Weinberg equilibrium for 31 of the

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TABLE 1. Summary data for 68 microsatellites developed from red drum (*Sciaenops ocellatus*) genomic libraries. The PCR primer sequences are forward (top) and reverse (bottom). Primers developed from a single clone are designated with the same letter as subscript.[a]

Micro-satellite	PCR primer sequence (5′ → 3′)	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N_A	Range in allele size (base pair)	H_o/H_e	P_{HW}
Soc 9	AACATTTCCATCACGTATTTATCT TCCACATGAACACCAGTGCAGTTC	$(AT)_{27}$	233	58	12	4			
Soc 11	GCCGAGTCACGAAGGAACAGAGAA TGTCGTCTCATCTATCTCCATCTC	$(GA)_{11}$	219	62	45	14	217–240	0.733/0.721	0.713
Soc 12	GCACCATCTTGCCACTGATGAATT GGGCTCTTACAACTCGTTTCAGAT	$(GT)_7$	187	62	12	2			
Soc 19	GGGTACAACTAAACAGACACAATA TTTGAAAATGTTCCTGTGAATCAC	$(GATA)_{16}$	229	58	45	21	195–267	0.844/0.898	0.019
Soc 34	TCTTTTTCTGTCTTTCAGGTAAGC AACCGTCTTCAACAAGGCTGTGAC	$(GT)_8$	176	58	12	1			
Soc 35	TGTCCCATCAATCAAGCAGACTCT CTCTACCTCACACTCCTCAAAGTT	$(CT)_5$ $(CA)_9$	262	62	12	19			
Soc 44	GAGGGTGACGCTAACAGTTGA CACAGCTCCACTCTGATATG	$(CA)_{22}$ $(GT)_5$	230	62	43	6	211–271	0.907/0.930	0.363
Soc 49	GTTGCCTTCTGACAATACACTGTT CCGGCTCGCCTTGAAATGAATGAT	$(CA)_{24}$	237	58	12	6			
Soc 50	CCCGTGATTTTAGGCTCAGATA CCTTTAGAGTGCAGTAAGTGATTT	$(GT)_7$	183	58	12	3			
Soc 60	TCTATTGAAGCCTGTAAGTTAGTT CAAGGAAGGAGTGGGGAATGACAA	$(AGG)_8$	155	56	45	6	151–163	0.511/0.570	0.752
Soc 77	TAGCCCTTTGCCTCTCAGAA ACCCATAATGGACCTATTTC	$(TG)_{22}$	147	58	12	1			
Soc 83	TGCTGTAATTGAAAAGCAGTGTAC AGCGGAACTAGAATTGGTTTTATA	$(TG)_{19}$	130	56	45	6	114–142	0.867/0.826	0.682
Soc 85	TTTTGGACCTACACTAGAGTAGC CGTGGGAGACTAGCGATGTAGAT	$(AC)_{17}$	104	58	45	5	80–122	0.822/0.869	0.105
Soc 86	TCTGCTTCTATATTTCCACTTTTT TTACACGGTGCCGCTCACAG	$(TGTC)_9$	135	56	12	1			
Soc 99	CACCCACTGACACACACATACAC GGAACCAATATGTCTGCCATGAT	$(CA)_{29}$	185	62	45	1	131–209	0.933/0.923	0.662
Soc 105	TGGGGAAGAAAAACAGGGAG AAACCCCTGCATCTCTCTAAAC	$(AG)_5$	191	56	12	1			
Soc 125	CCGCCGGCCACTCTGAGGACTCAT ACACTTGCGCTCATACAGTTAGCT	$(TG)_{10}$	124	56	12	7			

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TABLE 1. Continued.

Microsatellite	PCR primer sequence (5′ → 3′)	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N_A	Range in allele size (base pair)	H_o/H_e	P_{HW}
Soc 133	CATTTGGACCATCGCTACTGCTG CTTGGCATTTCCAGACATCACTG	$(TGC)_{10}$	205	56	12	3			
Soc 137	AGGATCAGTCTCCGTTTGT ACAGACAGATTCACAGCCAGAC	$(TGTC)_8$	223	58	12	1			
Soc 138	CTGGAGCTTTTCCCTTTCTGT TGGGAGGAGAAGGCAGGAAGG	$(TGTC)_6$	91	58	45	8	77–123	0.889/0.820	0.203
Soc 140	GGTGCAAACACAGCCATACAGT GCAAAATCGAAGACCGAGTTTAG	$(CTGT)_8$	142	56	45	5	132–144	0.778/0.623	0.280
Soc 156	CCTCTCCTTTCTCCATCAGTGC AGCCCGGCTGTCATCTCCTGTA	$(CCT)_6$ $(TCC)_4$	182	58	45	12		0.533/0.453	0.018
Soc 177	TCCAAGTATTTGACTGTTGTAGC AGATTACGAGTTTAGGTAGACAT	$(TAGA)_{10}$	192	58	12	12			
Soc 201	GGAGGAACTGATGAGGGCAGTGT GCACAACACACCTCGCTATATC	$(CCT)_6$	229	58	45	4	224–243	0.600/0.678	0.310
Soc 204	ACAGCAGTACCTGCCCAAAACTG TCCCCTTCGTCTTCTTCCACTTC	$(CTG)_{12}$	193	58	12	14			
Soc 206	GTTTCCCACATCCCCCAACC AGTTTGGTCGCTTTAAAGGC	$(GCAC)_5$	257	58	45	4	249–265	0.578/0.548	0.028
Soc 232	AGGGCACAGTTGCATCTCTG CCCATCCTCAAGGCAGAAC	$(AGAC)_4$	184	56	12	1			
Soc 243	GACGGGGATGCCATCTGC AATGCGAAAAAGACGAAACAGT	$(CCT)_9$	106	56	45	6	94–106	0.733/0.753	0.243
Soc 247	AGGCGCTGTTTCTGAATTTC TGGGAGTTTTTTATGGTGGT	$(TAT)_7$	210	56	12	2			
Soc 252	GCTCCAATTAGTCCCCATTC GCGGGCTTTCTCTAGTCACA	$(CA)_{10}$	114	62	12	19			
Soc 400	TGCCATTGTCATTCTACAGAGC TTATAGTGGGGTGAGTGTTTGA	$(CA)_{19}$	253	52	45	7	245–266	0.622/0.719	0.688
Soc 401	ACGTCTTAATCGGTCTCTGTCC ATCTCTGTGTGAAAGGAAAACA	$(TG)_{14}$	174	52	45	6	174–206	0.733/0.848	0.463
Soc 402	CATATTTAACGAGCGACATAGC AAACAGATGAAGCACCTGGACT	$(CA)_{20}$	149	52	44	5	134–164	0.818/0.878	0.262
Soc 403_A	AGGGAAATGGTTGGTGAAGTAG GTCTGGACCTGTTTGTTGAGAG	$(TG)_{36}$	272	58	6	11	272–310		

TABLE 1. Continued.

Microsatellite	PCR primer sequence (5′ → 3′)	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N_A	Range in allele size (base pair)	H_o/H_e	P_{HW}
Soc 404_A	AGACCCTTTTGTTGATTTCATA ATGACTGCACCATTTCAAAAAG	$(TG)_{23}$	168	52	45	9	150–212	0.778/0.904	0.018
Soc 405	CTTAGCCTTTTGTTTAGTTTCC CACACTCATGGTCACTCCTCTC	$(CA)_{12}$	189	56	8	5	189–217		
Soc 406	TAGGGGGTAAGGTAGGATGATG GAAGAGCAGTGACGCTATCAAT	$(TG)_{10}$	165	52	8	1			
Soc 407	AAAGTCTGCCTCTTACAGCTTC GAGTTAAAGCGTGTGCTAGTCC	$(CA)_{13}$	147	56	43	6	139–157	0.907/0.843	0.341
Soc 409_B	TTTATCTGCTCTGTGTGGAAGT ATCTATTTGTCGGTTTCTCTGC	$(TG)_{11}$	323	52	8	7	323–367		
Soc 410_B	GTACCAAGTCAGCCAGTGTCAG TCTCTGTGTCCCTCTGTGTTTG	$(TG)_{17}$	318	56	43	7	306–344	0.721/0.810	0.169
Soc 411	TCTGCCTCTTACAGCTTCAAGG CTTGTTGAGTTAAAGCGTGTGC	$(AC)_{13}$	149	54	7	6	147–163		
Soc 412	CACAGAAACTCAGCTCGAGACC AGGAAGAATGTACAAGGTGTTC	$(AC)_{13}$	114	49	44	6	102–168	0.818/0.906	0.003
Soc 415_C	CTCAGCACCCTCAGACATATGG CACAAGTTAAGTGGTATCGAGT	$(TG)_{15}$	193	52	45	6	187–235	0.667/0.709	0.583
Soc 416_C	CTCGATACCACTTAACTTGT ATCGACATAATCTGGCACCA	$(GA)_{38}$	159	49	45	6	141–181	0.733/0.851	0.368
Soc 417_C	CTTACGTGATAAAGTGTGGGTGA ATATGCCAGTAATCCACCGAAG	$(AC)_{24}$	96	49	45	4	86–112	0.778/0.756	0.278
Soc 418	GTTTTTCTGGCATTTATGGATG TGAGGTTATCAAACACCTGCCCACT	$(TG)_{24}$	288	52	8	22	272–294		
Soc 419	ATTTAGCCAACTGCTCCGCTCA GAGTGCGTGGTGTAGGGGGGTA	$(AC)_{20}$	246	56	42	6	238–260	0.929/0.847	0.909
Soc 421	CTCACTGCTCCCTCGTCACACG CTGTGACAGGATGCGGCTTTTC	$(TG)_{34}$	172	56	8	10	138–188		
Soc 422	CTGAAGGGATGGCAATGTTGATTGG ATTCTCTGGGTTTATGGGATGT	$(TG)_{34}$	372	56	7	6	360–374		
Soc 423	GTCACCGCACCATGATGGAGAT TACCACTTACACTCAGCAGGTG	$(CA)_{26}$	202	54	45	6	172–208	0.889/0.881	0.126
Soc 424	CACTCTTCATCCCTCACTCGTC TTCGATGGGTGACAGCGTCAGG	$(CA)_{15}$	208	56	45	9	204–230	0.844/0.840	0.333

TABLE 1. Continued.

Microsatellite	PCR primer sequence (5′ → 3′)	Repeat sequence of cloned allele	Size of cloned allele (base pair)	AT	N	N_A	Range in allele size (base pair)	H_o/H_e	P_{HW}
Soc 425	ACACCGCATTGCCCACCAGGAA CGAGTTTATCCTTCACGCTTG	$(CA)_{14}$	150	54	8	2	149–150		
Soc 426	GAGAGGACGTGAGCTGCTGA TGAGAAACAGAAACAGAAGGT	$(CA)_{11}$	142	52	8	4	138–152		
Soc 428	GACATCGCATTTGTCTACAGAGTCG AACTCCCAGTCATAATATCCCTTT	$(TG)_{38}$	229	53	45	8	172–242	0.956/0.946	0.256
Soc 429	AAAAATTCTGCCTGCCTGTG TTAAGAGCAACCTCCGTCTC	$(TG)_{12}$	128	52	8	4	124–132		
Soc 430	TAACAGTCCCTAAACAGGTT GTTTCTCCTCCCCTTTCCTC	$(TG)_{23}$	277	52	8	10	265–339		
Soc 431	GACACGCTGTGGTAGATGAAAACG TGTATATTAGTTGGCAAGGCAGAG	$(TG)_{29}$	172	53	8	8	151–180		
Soc 432_D	TTTAGGCTACGTCTGGAGGCACA GTGTGTTTGAGGGTCAGCGTAC	$(AC)_{16}$	108	52	45	5	98–118	0.867/0.808	0.868
Soc 433_D	AGTACGCTGACCCTCAAACACA TTCTCTTTGCCTCCTTTTTCCCTGA	$(TG)_{16}$	100	52	45	6	84–102	0.867/0.828	0.194
Soc 434	GACACTCCCAGATATGCTGA TCCTTGTTTATCTTGGTGCTGT	$(CA)_{23}$	197	52	7	7	169–219		
Soc 435	AACTGGAGCCTGACTCACTGC GTGATAACTCTCTTTTCTTGTG	$(AC)_{22}$	179	49	8	1	179		
Soc 437_E	CTACTTTCTAGTCTTTGCTCCACT GTCAAACGCTATTTTTTCCAGT	$(TG)_{36}$	296	54	5	7	296–330		
Soc 438_E	AATACAGCTAACTCGAAA ACTGCACCATTTCAAAAACGCCTCT	$(TG)_{24}$	144	49	7	6	132–154		
Soc 439	ACTCTCGTCCCACTTACCACA TATGTTTGCATATAAGCTCA	$(TG)_{17}$	103	49	6	4	91–105		
Soc 442	TTTGTTGGCAATAAACTGCGAGA TTCTTAATACGTGCCCCGACT	$(TG)_{30}$	195	52	8	8	179–199		
Soc 443	CACAGGAGGAGTTTGTCCAAT ATGTTTCGGTTTTCGTTTGCTC	$(TG)_{15}$	202	52	7	11	206–242		
Soc 444_F	TGAACTAATCCAGCCACAGATG CACAGCCGATTAAAGAGAGGGAAT	$(TG)_{17}$	161	52	45	3	161–165	0.600/0.504	0.526
Soc 445_F	ATACAAAGGGACTCTCATACTCTC TTTTAATCCCATTACAGCTTT	$(TCC)_{10}$	156	52	45	7	134–166	0.778/0.805	0.129

[a] AT = annealing temperature; N = number of individuals assayed; N_A = number of alleles detected; H_o/H_e = observed/expected heterozygosity; and P_{HW} = probability of conformity to Hardy–Weinberg equilibrium.

polymorphic microsatellites based on a sample of 45 wild-caught adults. We anticipate that the microsatellite markers will be useful for a variety of studies, including monitoring and assessment of red drum stock enhancement.

Details on genomic library construction, ligation of size-selected (200–1,200 base pair) fragments into cloning vectors, and transformation into *Escherichia coli* competent cells may be found in Turner et al. (1998) and O'Malley et al. (2003). A total of 14,080 clones were hybridized with cocktails of oligonucleotide probes, and 393 positive clones have been sequenced. Thus far, 204 clones that contained microsatellite motifs have been isolated. The PCR primers were designed from sequences flanking the microsatellites by using the program Oligo™ (Macintosh version 4.0, National Biosciences), and optimization of PCR protocols was carried out on a panel of DNAs from 12 individuals. The PCR was performed in a 10-μl volume containing 1 μl (100 ng) of DNA, 1 μl of 10× reaction buffer [500 mM KCl, 200 mM Tris–HCl (pH 8.4)], 1.5 mM $MgCl_2$, 2.5 mM of each deoxynucleoside triphosphates, 5 pmol of each primer, and 0.5 units *Taq* DNA polymerase (GibcoBRL). The PCR thermal cycling consisted of an initial denaturation at 95 C for 5 min, followed by 30 cycles consisting of 45 sec at 95 C, 45 sec at the optimized annealing temperature (Table 1), 1 min at 72 C, and a final extension of 10 min at 72 C.

The PCR primer sequences, repeat sequence and size (in base pairs) of cloned alleles, optimal annealing temperature, number of individuals assayed, and number of alleles detected for all 68 microsatellites are given in Table 1. The range in allele size is given for a subset of 48 microsatellites. The entire set of 68 microsatellites includes 51 di-, six tri-, and eight tetranucleotide repeat motifs; three microsatellites are complex repeats (i.e., a combination of different repeat motifs). Sixty of the microsatellites were found to be polymorphic; the average number of alleles per (polymorphic) microsatellite was 7.5 (range = 2–22 alleles). Genotypes for a subset of 31 microsatellites were acquired from 45 adults sampled from offshore waters along the Texas coast and held by Texas Parks and Wildlife (TPW) as broodstock for the TPW stock enhancement program. Estimates of observed and expected heterozygosity (gene diversity) and results of tests for conformity of genotype proportions to Hardy–Weinberg expectations for these 31 microsatellites also are given in Table 1. Estimates of observed and expected heterozygosity were computed using GENETIX v. 4.05 (Belkhir et al., 1996–2002); probability of departure from Hardy–Weinberg equilibrium (P_{HW}) was assessed using a Markov chain method (Guo and Thompson, 1992), as implemented in GENEPOP v. 3.3 (Raymond and Rousset, 1995) and using 5,000 dememorizations, 500 batches, and 5,000 iterations per batch. The average number of alleles for these 31 microsatellites was 12.6, and the average expected heterozygosity (gene diversity) was 0.784. These values are intermediate between those typically found in marine and anadromous fish (DeWoody and Avise, 2000). Following (sequential) Bonferroni correction for multiple tests performed simultaneously (Rice, 1989), genotypes at all 31 microsatellites did not differ significantly from Hardy–Weinberg equilibrium expectations. Tests that were significant before Bonferroni correction included microsatellites *Soc* 19, *Soc* 404, and *Soc* 412 (heterozygote deficiency) and *Soc* 156 and *Soc* 206 (heterozygote excess). This set of microsatellites should prove to be an extremely powerful tool for future studies of red drum population structure and for parental assignment (kinship) in studies monitoring the results of red drum stock enhancement.

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