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PERMANENT GENETIC RESOURCES

PCR primers for 100 microsatellites in red drum (*Sciaenops ocellatus*)

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Abstract

One hundred nuclear-encoded microsatellites from a genomic library of red drum (*Sciaenops ocellatus*) were isolated and characterized. Eight microsatellites had tetranucleotide motifs; 92 had dinucleotide motifs. The average number of alleles per microsatellite (sample of 22–24 fish) was 17.7 (range = 2–30); gene diversity averaged 0.796 (range = 0.227–1.000). Following Bonferroni correction, genotype frequencies at 90 microsatellites did not deviate significantly from Hardy–Weinberg equilibrium expectations. Occurrence of null alleles was inferred at 15 microsatellites; alleles differing by only a single base were observed at 11 microsatellites. The microsatellites developed should prove useful for population-genetic studies of 'wild' red drum and in construction of a genetic map.

Keywords: Microsatellites, red drum, *Sciaenops ocellatus*

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Nuclear-encoded microsatellites are useful for a variety of studies, including (i) assessment of population structure and genetic effective size (Saillant & Gold 2006), (ii) parentage assignment and identification of quantitative trait loci (QTLs) (Liu & Cordes 2004), and (iii) 'anchor' loci in construction of genetic maps (Silver *et al*. 1991). Added to the 68 already developed primer pairs for red drum microsatellites (Saillant *et al*. 2004), the 100 developed here should provide a firm basis for a red drum genetic map that should prove useful in future studies of red drum.

Whole genomic DNA was extracted from ethanol-preserved fin clips, using a DNeasy gel extraction kit (QIAGEN). DNA was digested with *Dpn*II (NEB), and 500–1500 base-pair (bp) fragments were dissected from an agarose gel, purified with a DNA gel extraction kit, and ligated in a P-Bluescript II KS-plus plasmid. Transformation with XL-Gold Ultracompetent cells (Stratagene) and screening of transformants were performed as described in Renshaw *et al*. (2006). A total of 48 000 clones were spotted in a 4 × 4 array onto two identical Hybond H + nylon membranes (22.5 × 22.5 cm, Amersham), using a Q-bot (Genetix). DNA from each clone was fixed to the membranes as outlined in Renshaw *et al*. (2006) and each clone was spotted twice to minimize the possibility of false positives. One of the membranes was hybridized with ^{32}P-labelled tri- and tetranucleotide probes [$(CAA)_8$ $(GAA)_8$ $(TAA)_{13}$ $(GATA)_9$ $(CATA)_8$, and $(GACA)_8$], while the other was hybridized with ^{32}P-labelled dinucleotide probes [$(CA)_{13}$ and $(GA)_{13}$]. A total of 104 positive clones were identified on the membrane probed with tri- and tetranucleotide repeats; 459 positive clones were identified on the membrane probed with dinucleotide repeats.

A total of 523 positive clones (64 tetra-/trinucleotide and 459 dinucleotide repeats) were inoculated into frozen glycerol stocks arrayed in 96-well plates with 1 mL of Luria Broth selective media (ampicillin) and incubated over night at 37 °C. Plasmid DNA was isolated with a BioRobot 8000 (QIAGEN). Miniprep DNA was quantified and normalized, and both strands were sequenced using primers 555 and 837 (Makova & Patton 1998) and ABI PRISM BigDye Terminator version 3.1 (Applied Biosystems). Sequencing products were separated and visualized on an ABI 3100 (Applied Biosystems). A total of 197 sequences were edited, the vector sequence was removed, and repeats were identified using Sequencher 3.0 (Gene Codes Corporation); seven of the sequenced clones (197) contained no repeats. Primer design was performed by using PRIMER 3 vs. 0.3.0 (Rozen & Skaletsky 2000). Primers generated by PRIMER 3 were assessed further using NETPRIMER

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(http://www.premierbiosoft.com/netprimer). Primers were imported to AMPLIFX 1.4.0 (available at http://ifrjr.nord.univ-mrs.fr/AmplifX-Home-page?lang=en), where simulations of PCR amplification of designed primer pairs to the imported target sequence were performed.

A total of 122 primer pairs were designed and used in genotype assays of 24 red drum individuals obtained from Galveston Bay, Texas. Total genomic DNA was extracted from ethanol-preserved fin clips. PCR amplifications were performed with a PTC-200 thermocycler (MJ Research) or a MyCycler thermocycler (Bio-Rad), using unlabelled 5′ (forward) and 3′ (reverse) primers and a fluorescently labelled, 21 bp 5′-tail-sequence primer (as described in Boutin-Ganache *et al*. 2001). The 5′-tail-sequence primer was arbitrarily chosen from the microsatellite primer sequence CATR8 used to amplify DNA from the Madagascar periwinkle *Catharanthus roseus* (Shokeen *et al*. 2007). Because only the 5′-tail-sequence primer needs to be labelled, the approach significantly reduces cost when evaluating multiple primer pairs. The unlabelled 5′ forward and 3′ reverse primers and the 5′-tail-sequence primer were purchased from Invitrogen. The 5′-tail-sequence primer was labelled with either with NED (Applied Biosystems) or with FAM (Invitrogen). PCR amplifications were performed in a 10 μL reaction volume containing ~100 ng DNA, 1× reaction buffer (50 mM KCl, 10 mM Tris, 1% Triton-X 100), 0.5 μM of each primer (tailed forward, reverse, and 5′-tail-sequence), 2 mM $MgCl_2$, 200 μM of each dNTP, and 0.1 U *Taq* DNA polymerase (Invitrogen). All microsatellite amplifications were run under the same PCR conditions, using a PCR protocol with a three-step decrease in annealing temperature (with the same denaturation and extension conditions). The PCR protocol consisted of initial denaturation at 95 °C for 3 min, 10 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 45 s, extension at 72 °C for 1 min; 10 cycles of annealing (45 s each) at 56 °C, 15 cycles of annealing (45 s each) at 52 °C, and final extension for 10 min at 72 °C. PCR products were separated and visualized on an ABI 377 automatic sequencer. Alleles were sized using the GeneScan® 400 ROX-Size Standard (Applied Biosystems) and GeneScan 3.1.2® (Applied Biosystems); allele-calling was performed using GENOTYPER® software, version 2.5 (Applied Biosystems).

One hundred microsatellites (eight with tetra-nucleotide motifs and 92 with dinucleotide motifs) were amplified successfully and reliably scored for genotypes. The remaining 22 primer pairs designed failed to amplify product or product could not be scored reliably. *Soc*500 and *Soc*506 were designed from the same clone, while the remaining primer pairs were designed from different clones. Searches in GenBank, NCBI (nucleotide blast) did not reveal any matches with target sequences of previously designed primer pairs for red drum. Number of alleles, observed and expected heterozygosities, tests for conformity to Hardy–Weinberg equilibrium expectations, and tests for linkage equilibrium were performed using GENEPOP (Raymond & Rousset 1995). Evidence for occurrences of null alleles and/or large allele drop-out was explored using MICRO-CHECKER (Van Oosterhout *et al*. 2004).

Primer sequences and summary genotypic data are presented in Table 1. The number of alleles detected per microsatellite varied from two to 30; the average gene diversity varied between 0.227 and 1.000. Significant deviation from

Table 1 Summary data for 100 microsatellites developed for red drum (*Sciaenops ocellatus*)

Microsatellite	GenBank	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
*Soc*500	EF609016	GTCCGTGCGAGTGTGTATTAGA GCCAGAGAGAAAACGAGAAAAG	$(CA)_{27}$	162	24	16	157–195	0.833/0.905	0.180
*Soc*501*	EF609017	CTGTTGGTGTTGTTGACTCTGA GCACACAAACTACAACTGAATCTG	$(GACA)_7$	211	22	8	203–239	0.227/0.823	**0.000**
*Soc*504	EF609018	CCAGTGTCTCCAGTGTTACCAGT CCTGCTCTACACATCGGCTCGT	$(GACA)_6$ $(GACA)_8$	160	23	8	176–208	0.956/0.849	0.639
*Soc*505	EF609019	ATGGCACTGGTATCAAGAAT GGAAATGGGAGCAAAAACA	$(GATA)_{14}$	105	24	14	114–178	0.833/0.926	0.092
*Soc*506	EF609020	CACTCCTACAGCCTAGTTGTTA GCTCTGCTTGGTATCCTTGG	$(AGCA)_6$	148	24	2	164–168	0.333/0.383	0.598
*Soc*507	EF609021	GCTGAGCAGAAAAGATGAGATAG CAGAGAGCCCAATGAAGGTC	$(GATA)_{24}$	318	24	18	291–379	0.875/0.949	0.215
*Soc*508	EF609022	GCAGCACATTTCAGCACAC TAATGCCCCTGTTATCTATCTA	$(GATA)_{18}$	148	24	9	136–172	0.917/0.868	0.597
*Soc*509*	EF609023	TTGACATCGCAGGAGTGAGA GGAGACAGACGGATAAGCATA	$(GATA)_{11}$	192	24	8	206–238	0.542/0.756	0.029
*Soc*510*	EF609024	AGATGCCAACACCTCTAAAACT ACAGACCACTCCGACTCAAA	$(GATA)_8$	256	24	7	269–297	0.458/0.637	0.110
*Soc*512	EF609025	CCATCCCTCGCTCTCTCTT GGCTCACCTAATGAATCCAG	$(GA)_{17}$	104	24	9	122–138	0.750/0.831	0.222

Table 1 *Continued*

Microsatellite	GenBank	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
*Soc*513†	EF609026	ATGACTCCCAGAGAGACAAAGA CCTCAGCACAAGATAAAGACAA	$(CA)_{34}$	287	24	12	291–311	0.667/0.811	**0.000**
*Soc*516	EF609027	GCAGACACAAAATGTTCAAAGCA GGAAGACTCACGGAGCAGGTT	$(CA)_{11}$	157	24	5	169–189	0.750/0.566	0.058
*Soc*521	EF609028	CAGAAGGGCTACCAGGACC ACAAGGATTTTTACTGTTGATACT	$(GA)_{28}$	166	24	18	161–207	0.917/0.931	0.829
*Soc*522	EF609029	CTAGTGGATCTGCTTGATGCTTT GCACACGTCTGAGGAGTGAA	$(CA)_{13}$	116	24	10	124–150	0.750/0.798	0.207
*Soc*523	EF609030	TGACACTTCAAACATCCTCCTC GACCTCCCAAAGTCTGTAATCA	$(CA)_{16}$	100	24	5	106–120	0.667/0.751	0.143
*Soc*524	EF609031	TGTTTCCCTGCTGCTGTAATG CCACACACCGACTACAATGAA	$(CA)_{25}$	128	24	18	116–172	1.000/0.916	0.841
*Soc*525*	EF609032	CACTGTGGGGACATTTAGAGG GGGTGTGACAAACTCAGGGAC	$(CA)_{14}$	108	24	7	119–137	0.417/0.734	**0.000**
*Soc*526	EF609033	CTTCCTCCTCCACTTCAACA GTAGTGTTTGTGACAGCAGGAC	$(CA)_{16}$	219	24	16	229–273	0.875/0.933	0.737
*Soc*527*	EF609034	CCAAATGTCAAGCCTGCTC GCTGTGAAAATCTCACCACACTT	$(TG)_{16}$	193	24	13	204–236	0.708/0.908	0.045
*Soc*529	EF609035	ATGTGTGACCAGACCCAGTTC CGTCAACAGGAAACACTAACAG	$(CA)_{20}$	171	24	15	190–222	0.833/0.717	0.320
*Soc*530	EF609036	CTTGGACCTGTATCACAGATGGA GTTCTCCTTCTCATCTTCAGCAT	$(CA)_{14}$	203	24	12	217–247	0.792/0.839	0.060
*Soc*532	EF609037	GAGTGCTCACAAAGTGCCAG GTGCCAGATAGATGCTGACG	$(CA)_{14}$	124	24	12	130–162	0.833/0.857	0.030
*Soc*533	EF609038	CTGGACAGTGAAAACTTGAACA GTTAGGCTAATACACTCTTGGTG	$(CA)_{25}$	101	24	13	102–130	0.708/0.838	0.073
*Soc*535	EF609039	CGAGCGTAAACAAAGCACCTA GACTCACTGTAACACCACCAATCT	$(CA)_{16}$	122	24	8	126–140	0.750/0.844	0.858
*Soc*536	EF609040	GCCACAAAAGTCATACTGCTA GTGTGAAACTTGTAAACTTGAATG	$(CA)_{26}$	166	24	9	152–186	0.833/0.824	0.358
*Soc*538	EF609041	CCAGCATATTTTGAGCAGC TGAAGGTTTTCCCCGTAGT	$(CA)_{17}$	182	24	11	196–218	0.875/0.847	0.631
*Soc*540	EF609042	GCTCGTTATCTGTATCAAAGGTT CTTGAAGCAGCGTTTGGAT	$(CA)_{13}$	154	24	6	174–184	0.667/0.720	0.723
*Soc*541	EF609043	CCGAATAGATAGAGCCGTCACA GCTGCTGAGGTGTTGATGAAA	$(CA)_{28}$	193	24	13	171–209	0.750/0.869	0.386
*Soc*542*	EF609044	CTGTTGGAAATACCAGGAAA CAGTAATAGACTCTGATTGTGCTC	$(CA)_{41}$	135	24	20	102–176	0.792/0.948	0.113
*Soc*543	EF609045	GAGCATTCAGGAGCCACCAT CCATCTGTGCCTAACGACCA	$(CA)_{16}$	189	24	16	206–282	0.833/0.837	0.762
*Soc*544*	EF609046	CAGACCTGCTGTGTGCTTTTC TCTTGATTTGAGAGGGATAGTGC	$(CA)_{26}$	121	24	25	127–259	0.792/0.969	0.010
*Soc*547	EF609047	AGTCACAAATACACAGGATGAAAA AGGCATGGACAAACTGGTCA	$(CA)_{16}(TA)_{11}$	197	24	8	208–224	0.792/0.773	0.696
*Soc*548	EF609048	CGCACACACAAAAACAGTGAG CCATCGGTCCAGTATGAAGTC	$(CA)_{13}$	160	24	9	170–186	0.958/0.874	0.849
*Soc*549*	EF609049	TGAGTGTCTAAAGTCGCATT CCATTTGAGTCCCGTAAC	$(CA)_{29}$	236	24	22	219–283	0.792/0.949	**0.000**
*Soc*550	EF609050	CGCAGACAGACAGCCTCTAT CATCCTCTCCTCAGTGTAGCC	$(CA)_{26}$	216	24	11	202–236	0.958/0.886	0.724
*Soc*551	EF609051	TCAACCACACTCAGGTCCAG GCAACACAAAGCACTCACAAA	$(CA)_{10}$	212	24	16	230–282	0.792/0.789	0.253
*Soc*552†	EF609052	ATCAGGAACCCCAGCAATC TGGTGACAAGGTGGCAGAAT	$(CA)_{26}$	153	24	12	153–185	0.875/0.885	0.255
*Soc*553†	EF609053	CAATCACACTCCGACACACTC GCATGGGTAGAGAGCTGGTG	$(CA)_{13}$	241	24	12	250–270	0.708/0.864	0.012
*Soc*554	EF609054	GAAAGTAGTCCAACATCCAAGT AAATGCCAGTTTTCTCAGG	$(CA)_{40}$	225	24	21	204–276	1.000/0.957	0.886
*Soc*555	EF609055	TACAAGCTGCCGAAACACAC AGAATGAGAGCCGACAGAGG	$(CA)_{15}$	183	24	7	195–207	0.917/0.793	0.024
*Soc*556*	EF609056	CAGAACAGACACACCAGCAG CAAACACAGTTAGACAACAGAGA	$(CA)_{18}$	203	24	14	209–251	0.542/0.885	**0.000**
*Soc*557	EF609057	AGTCCTCGTGTGTTTTGGTAT GCTGGTGGTTTGTGATTGAT	$(CA)_{16}$	197	24	3	215–219	0.333/0.393	0.667

Table 1 *Continued*

Microsatellite	GenBank	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	N	N_A	Size range	H_O/H_E	P_{HW}
*Soc*558	EF609058	CAGTGAACAGGAACGACTTTTAGA GACCCATCAACCTCCAGCA	$(CA)_{18}$	168	24	12	187–217	0.958/0.913	0.472
*Soc*559	EF609059	TGTGGGACAAGATGTGAAAT TTACCTTGGAAACGGTGTGA	$(CA)_{31}$	221	24	26	233–311	0.958/0.964	0.911
*Soc*560	EF609060	ACGGCTCCCTACACATTACG GTCCTCGGCACAGAAAGTTG	$(CA)_{15}$	176	24	6	196–206	0.625/0.729	0.089
*Soc*562†	EF609061	CTGAGGAAACACACACACGAA CACAAAATCAATGGCTGCTG	$(CA)_{11}$	247	24	5	259–272	0.667/0.621	0.112
*Soc*563	EF609062	ATCCGTTATGTGGCTTCA CGCTTTCAAATGTGGTCTT	$(CA)_{42}$	166	24	18	141–201	0.792/0.901	0.145
*Soc*564	EF609063	TGCCAGCATCCGTCTACTCA AAGAGCCGACCAATCAGAGAG	$(CA)_{15}$	171	24	7	187–201	0.708/0.677	0.993
*Soc*565	EF609064	CCTCACCCACCCGATACAT CAGAATCAGAGAGAGAGAATCAGGA	$(CA)_{15}$	231	24	6	243–253	0.833/0.709	0.740
*Soc*566	EF609065	GGACAAGAGAAGCAGCAGACG TCATCACGTCCAAAGCTACG	$(GA)_{13}$	111	24	10	127–163	0.917/0.855	0.187
*Soc*567*	EF609066	ACACACACTCCCACAGATGAAT CGCTGCCCCAAAATAGAAT	$(CA)_{31}$	262	24	23	246–338	0.708/0.957	**0.000**
*Soc*568	EF609067	CGACTCTGATGGTGAATGTTGA GAGCGTTCCCTGAAGAAGATG	$(CA)_{17}$	197	24	12	196–228	0.875/0.885	0.817
*Soc*569	EF609068	CCCTGTGTGTCTGTTTCCTTTG TGTGAGTGGTGGTGAGTGATGTT	$(CA)_{13}$	196	24	6	212–222	0.708/0.683	0.787
*Soc*570*†	EF609069	GCGTCATCTTCTCCACCTCT ACGACTCCTCCCATACTCTGTT	$(CA)_{40}$	178	24	30	173–403	0.792/0.975	**0.000**
*Soc*571	EF609070	CGTGAACTGAGCGGAGACA GGGAGTGTGTGAGAATGTGGA	$(CA)_{20}$	241	24	20	247–289	1.000/0.953	0.922
*Soc*572	EF609071	AGCACTATGTCTCCCAACCT ATCACCTTCAAATCAGTCTCTT	$(GA)_{32}$	130	24	10	134–154	0.875/0.872	0.752
*Soc*573	EF609072	AGAGCAGGAGGATGTGACTTC CTTTCTGGGAGGTTCAGCACA	$(CA)_{23}$	263	24	27	267–329	0.917/0.971	0.541
*Soc*574	EF609073	TTTCTCATAATCTGCCATACG TTGGTGGAGTGCTTCTTTCT	$(CA)_{21}$	211	24	8	220–242	0.750/0.760	0.949
*Soc*575	EF609074	GGACGCACCATCTCTCCATCT AGGCTTTGCTCTTTTCAGACG	$(CA)_{11}$	229	24	6	245–255	0.875/0.778	0.750
*Soc*576	EF609075	CCTGTCCGTCAAGCGTCTGT ATCCAAGTCTGAACTGTCACTGTT	$(CA)_{19}$	185	24	10	179–209	0.875/0.853	0.406
*Soc*577	EF609076	GGGATAGGCATCAGACACC CTTTGCGACAGCCCACTTC	$(CA)_{14}$	244	24	8	256–274	0.750/0.777	0.142
*Soc*578	EF609077	GATGGGAGACTGTGCTGATGAA ATGTTGCTCTGAATGCCTGCT	$(CA)_{20}$	296	24	14	306–344	0.958/0.894	0.824
*Soc*579	EF609078	GCTAACACACAGAGAAAAGAATG GGAAAGCAAACGGAGAGAGA	$(CA)_{32}$	295	24	17	275–321	0.750/0.886	0.026
*Soc*580	EF609079	CTGAGCCTGAACGCACATCAT GAACAAACAACTGTCACCTGCTG	$(CA)_{25}$	275	24	18	259–311	1.000/0.939	0.983
*Soc*581*	EF609080	CGTTATTTCTTGGACATAGTAGGC CAGTGAGAGCGACAGTATCTGCT	$(CA)_2\,(N)_2\,(CA)_7\,(N)_6\,(CA)_{24}$	210	24	19	221–275	0.792/0.933	0.012
*Soc*583	EF609081	CAAGGGAGGTGTTGCTGATTA TGAGTGTGGGTGGCTGATAGAT	(CA)12	111	24	3	125–129	0.417/0.370	0.143
*Soc*586	EF609082	GGGAAATGGACACAAAAGAAT CACCTGGGACCTTAGTCACTT	$(CA)_{35}$	180	24	22	162–228	0.833/0.917	0.146
*Soc*587†	EF609083	TGGAACATGAGGTGATAAGACG AGGGGATGTAAAGCCTGAAT	$(CA)_{23}$	249	24	17	242–307	0.875/0.912	0.101
*Soc*588	EF609084	TGAATGACTTGCTTTGCTGAA AATAACCCCCACCTCTCCCT	$(GA)_{22}$	191	24	12	182–220	0.917/0.878	0.520
*Soc*589	EF609085	ACGATGAGTGACGAGAACTTTTT CTTGAGCCAATAGCAGGATGT	$(CA)_5\,(N)_2\,(CA)_4$	137	24	3	149–159	0.458/0.414	1.000
*Soc*590	EF609086	CAATGGACAGTTTGAGAGTTC GAAACCCACACCAATCACT	$(CA)_{18}$	190	24	12	199–235	0.875/0.878	0.412
*Soc*592	EF609087	AGAAGGAGGTCAGGAGGCATT TCCCATTCACAAACACAAGCA	$(CA)_{13}$	119	24	8	128–150	0.708/0.779	0.173
*Soc*593	EF609088	TTGTCACGGAGGTTGTAGATG ACAGGAATGTGTCCCAAAATG	$(CA)_{12}$	94	24	4	106–112	0.667/0.630	0.614

Table 1 *Continued*

Microsatellite	GenBank	Primer sequence (5′–3′)	Repeat sequence	Cloned allele	*N*	N_A	Size range	H_O/H_E	P_{HW}
*Soc*594*†	EF609089	TCGTGCTCTGTCTCCGTTC TGACTATTTTTGCTTTTTACTTC	$(CA)_{13}$	175	24	5	193–232	0.333/0.543	0.045
*Soc*595	EF609090	TCATCATCCTCCTCCTTTTCTCT CTTTACCCACCCAAACTCCAT	$(CA)_{23}$	184	24	13	188–218	0.958/0.867	0.587
*Soc*596	EF609091	TTTACGCTCTGAAAACCTCTA AGGGTCGCTTTGTGTATCCA	$(TG)^5\,(AG)_{14}$	163	24	9	175–191	0.917/0.856	0.171
*Soc*598	EF609092	CTGGCGTCTGAACACTGC GTGAATCCCTGTGGGTGCT	$(CA)_{34}$	214	24	25	197–275	0.958/0.968	0.334
*Soc*600†	EF609093	CTAGTGGATCAGTTTGACTTCA ATGAGCCATTATACAGGAACC	$(CA)_{11}$	201	24	9	211–236	0.750/0.834	0.446
*Soc*601	EF609094	CTTTGGGACAACAGAAATGC TCCAGCAAGCAGACAACAAT	$(GA)_{22}$	156	23	11	165–191	0.913/0.858	0.644
*Soc*602	EF609095	AGCAACCATTCTTCCCACAC CCATCACACACCAGGGTTAA	$(CA)_{13}\,(N)_2\,(CA)_5$	118	24	6	125–143	0.708/0.702	0.854
*Soc*604	EF609096	TGGGGAAAATGGAGACAGG CGTGACAGCCAATGAAAAAG	$(CA)_{19}$	227	24	11	243–269	0.792/0.876	0.147
*Soc*607	EF609098	GGTGAAGGATGACAGGAGAG CATACCAGCGTGACAAACA	$(CA)_{27}$	234	24	18	238–278	1.00/0.944	0.967
*Soc*609	EF609099	CCCGCATTAGACAGAAAAC ATGGGTATGTGTGGCTTACAG	$(CA)_{23}$	272	24	24	281–351	0.917/0.958	0.775
*Soc*610*†	EF609100	CCTGATTGTTTCACATTGGTTA GAAGGGAGGTGTTCCTGTTG	$(CA)_{32}$	163	24	25	134–209	0.667/0.969	**0.000**
*Soc*612	EF609101	TAATCCCTCTGTCACGGTCAT CTGTAAACGATGCGGCTGAG	$(CA)_{34}$	298	24	18	282–324	0.917/0.924	0.171
*Soc*613	EF609102	AGGGAGTGGCTCTTTGTCTGA AGGGGCATCCTGTCTGTTTG	$(CA)_{15}$	210	24	11	210–244	0.833/0.848	0.306
*Soc*615	EF609103	GATTACCAAGCACCAAGGA GCTCATCAGAAGGCTAACAGA	$(CA)_{26}$	97	24	25	80–150	0.917/0.965	0.016
*Soc*616	EF609104	TTCCTCTCTCCGTGTTTGTGTT ACTGGGCAGGTTTCTTCTGAC	$(CA)_{32}$	298	24	18	280–336	0.958/0.917	0.958
*Soc*617	EF609105	TGCCATGTTTGAACCTCAGA GAGGACAGAGGGTGTCACTCC	$(CA)_{44}$	290	24	7	281–311	0.667/0.782	0.371
*Soc*618	EF609106	ACCCGATAAGGAAACAAGCAGA CATCACCGTCCAACCAGAGAA	$(GA)_{17}$	107	24	8	125–145	0.708/0.796	0.523
*Soc*619	EF609107	GCGTTCTCTCTCTCGTGACAA GCTCTCCTGCGTCTCGTCTT	$(CA)_{19}$	170	24	10	172–202	0.875/0.819	0.189
*Soc*620	EF609108	CGTGTCAGCCACAATCTCCA AGCCCAGCCTAAACCTCTCA	$(CA)_{13}$	143	24	14	162–202	0.833/0.894	0.772
*Soc*621	EF609109	CACTCCTTCCACCTCCTCCT TGGTCTGCTGCTACTCTACTACTG	$(CA)_{12}$	152	24	8	169–193	0.875/0.784	0.781
*Soc*622	EF609110	CTGCCTAACATCCTTCTTTCTACT CCATAAACGCCACCAAACA	$(CA)_{29}$	251	24	14	244–276	0.875/0.864	0.439
*Soc*623	EF609111	CACTTTCACTTTCTGCCCTCA TCTGGTTTCTGGCTTTCATACA	$(CA)_{14}$	144	24	10	146–170	0.833/0.827	0.692
*Soc*624*†	EF609112	CACGCTGGTCTTTTTCTCAA CTGGGGTTATTTGTGTGTGC	$(CA)_{28}\,(N)_2\,(CA)_7$	158	24	22	135–192	1.000/0.977	0.032
*Soc*625†	EF609113	TACCCTCAGAAATGGTCGCTT GAGAGTGTTTACCCCGTGTGC	$(CA)_{12}$	109	24	11	118–133	1.000/0.919	**0.000**
*Soc*626	EF609114	ACTTTGAGCCAATGCTTTCC GTGAGCTGTGTATCCCTGTGC	$(CT)_6\,(CA)_9$	169	24	3	189–193	1.000/0.665	**0.000**
*Soc*627	EF609115	AGCAACGCATACAACCAGTG TCACGAGTAACGAAGCTGTGAT	$(CA)_{27}$	190	24	15	184–236	1.000/0.935	0.072
Soc629	EF609097	CCATCTCATCTCACTCTCATACTTG GGCGGGAGGAACAAAGAAT	$(CT)_{38}(N)_5(CA)_{15}$	180	24	14	148–178	0.750/0.892	0.554

*Microsatellites where occurrence of null alleles was indicated by analysis using MICRO-CHECKER.
†Microsatellites with observed alleles differing by only a single base.
GenBank Accession numbers for clone sequences; primer sequences are forward (top) and reverse (bottom); repeat sequence indicates repeat motif; cloned allele is size (in base pairs) of sequence in GenBank; *N* is the number of individuals assayed; N_A is the number of alleles detected; Size range refers to sizes (in base pairs) of alleles thus far uncovered — size includes the 21 base pair (bp) 5′-tail-sequence primer used for PCR amplification; H_O/H_E is observed and expected heterozygosities, respectively; P_{HW} represents the probability of deviation from Hardy–Weinberg expectations — significant *P*-values following Bonferroni correction are in bold.

Hardy–Weinberg equilibrium expectations, following Bonferroni correction (Rice 1989), was observed at 10 microsatellites (Table 1). None of the pairwise comparisons of microsatellites indicated significant genotypic disequilibrium. Evidence of null alleles was detected at 15 microsatellites; single base-pair shifts (i.e. alleles differing by only a single base pair) were observed at 11 of the microsatellites. The 77 microsatellites whose genotypic proportions did not deviate from Hardy–Weinberg expectations and where null alleles and one base-pair shifts were not detected should be suitable for population-genetic studies. Almost all of the microsatellites should be suitable for constructing a genetic linkage map.

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