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PERMANENT GENETIC RESOURCES

Isolation and characterization of 11 microsatellite primers for a temperate reef fish, the California sheephead (*Semicossyphus pulcher*)

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Abstract

Eleven microsatellites were characterized for *Semicossyphus pulcher* (California sheephead) using an enrichment protocol. The number of alleles varied from three to 14 for a sample of 40 individuals from two populations. Expected heterozygosities ranged from 0.311 to 0.891. All loci but one were in Hardy-Weinberg equilibrium. No evidence for linkage disequilibrium was observed. These polymorphic microsatellites will be useful for genetic diversity and connectivity analyses of *S. pulcher*.

Keywords: California sheephead, microsatellite, population structure, *Semicossyphus pulcher*, simple sequence repeat

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The California sheephead (*Semicossyphus pulcher*) is a protogynous hermaphroditic wrasse common to the temperate coastal waters from Point Conception, California, USA to Cabo San Lucas, Baja California, Mexico, including a disjunct population in the Sea of Cortez (Cowen 1985). Fishing pressure by both recreational and commercial fisheries has caused major changes in the abundance and size distribution of California sheephead in southern California. This pressure resulted in two major issues: declining stocks (California Department of Fish and Game, www.dfg.ca.gov/marine/sheephead2004/pdfs/summary.pdf), as well as an artificially biased sex ratio (Warner 1975; Platten *et al*. 2002). Neutral and highly polymorphic microsatellite markers are well suited to assess those issues (Waples 1998; Zane *et al*. 2002). Here we describe the isolation and characterization of 11 novel microsatellite loci (Table 1).

Genomic libraries enriched for microsatellite motifs were constructed by Genetic Identification Services (GIS, www.genetic-id-services.com; Chatsworth, California, USA). Libraries were built using a sample containing 100 μg of genomic DNA extracted from gill tissue of a single individual *S. pulcher* collected in Palos Verdes, California, USA. The sample was stored in 90% ethanol and extracted following a standard phenol–chloroform procedure (Sambrook *et al*. 1989). Libraries were enriched for CA, CATC, TACA, TAGA motifs. GIS sequenced 64 microsatellite-containing clones using universal M13 primers.

We tested 11 of these microsatellites, which were determined to have flanking sequences of length sufficient for primer design using DesignerPCR version 1.03 (Research Genetics, Inc.). Amplification reactions were carried out using a fluorescently labelled forward primer and a cold reverse primer (Sigma-Aldrich) in an Applied Biosystems GeneAmp PCR 9700 system in a total volume of 13 μL containing 1× PCR Mastermix (2.5 mM TAPS pH 9.5, 5.0 mM KCl, 0.2 mM $MgCl_2$, 20.0 μM of each dNTP, *Taq* 0.5 μ/μL, Thermo Scientific), 12.5 pmol of both primers and approximately 2 ng of DNA template. The following temperature profile was used: 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s and a final extension of 72 °C7 for 7 min. Forty individuals from two populations (20 from Guadalupe Island, Mexico and 20 from Catalina Island, California, USA) were genotyped to estimate allelic diversity and calculate average observed and expected heterozygosities for these 11 loci. All 11 primer pairs were polymorphic and successfully amplified for all samples of *S. pulcher* (Table 1). Microsatellite amplifications

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Table 1 Characterization of 11 polymorphic microsatellite loci in *Semicossyphus pulcher*

Locus	GenBank Accession no.	Primer label	Primer sequence (5′–3′)	Repeat range	Repeat range	Amp.	N_w	N_a	H_O/H_E	*P* values
SPU_A4	EU781576	H	F: 5′-TTCAGACACACGTCATCTTCAC-3′ R: 5′-CCATCAGTGAGTTACCGTAAGG-3′	$(CA)_{11}$	12–14	161–165	40	3	0.675 0.861	0.028
SPU_A7	EU781577	T	F: 5′-GGAAAGCCAATGAGGAAGTC-3′ R: 5′-CGTACACGTCTGGAGACCAC-3′	$(CA)_{13}$	12–25	259–297	40	9	0.475 0.528	0.600
SPU-A106	EU781578	F	F: 5′-CCTGGTGTTACTTCACATTGG-3′ R: 5′-AGGACAGCAACATCGACTTC-3′	$(CA)_{28}$	20–34	100–123	40	11	0.600 0.587	0.243
SPU_A109*	EU781579	F	F: 5′-AGTGGCTTTACTGAGTTTCAGG-3′ R: 5′-TTCCATCAGACAGGATTACATG-3′	$(CA)_{27}$	17–37	246–285	40	9	0.725 0.714	0.107
SPU_C7	EU781580	H	F: 5′-CGTTTATTGAGATGGATGACC-3′ R: 5′-TTTTCTACCCTTCTTCGATTTC-3′	$(CA)_{16}$	16–24	170–176	40	5	0.900 0.891	0.230
SPU_D2*	EU781581	F	F: 5′-GCACTCTCACTCAAAATGACA-3′ R: 5′-CAAGCCTGGTGTAATGTTTC-3′	$(TAGA)_9$	5–10	130–148	40	5	0.250 0.311	0.142
SPU_D101	EU781582	H	F: 5′-CTTCCGCGCTATAAAATAGTCC-3′ R: 5′-GAGGGGTTTGTATTAGTCCTGC-3′	$(TAGA)_{29}$	25–29	192–220	40	5	0.800 0.776	0.592
SPU_D106	EU781583	T	F: 5′-GCTGCTGAGGAAATGTAGAC-3′ R: 5′-TTTGGAGACAGAGGGAGAC-3′	$(TAGA)_{14}$	8–15	130–160	40	5	0.800 0.692	0.988
SPU_D113	EU781584	H	F: 5′-TCCTTCTTCACTGACTTAAACC-3′ R: 5′-CTTTCTGATTCTGCTCATGTC-3′	$(TAGA)_{21}$	14–21	215–243	40	4	0.625 0.590	0.288
SPU_D118	EU781585	T	F: 5′-GCAGCAGAGGTTAATTTTCAC-3′ R: 5′-AACAGCTATGACCATGATTAC-3′	$(TAGA)_{11}$	5–15	159–199	40	8	0.750 0.704	0.196
SPU_D120*	EU781586	T	F: 5′-TTGGCTTATCTGGACTTGAAC-3′ R: 5′-AAGGAGGAGGCTTACGTTAAG-3′	$(TAGA)_{14}$	4–20	215–267	40	14	0.825 0.852	0.497

Genbank Accession nos, primer label [(H, H_EX (green); T, TAMRA (yellow); F, Fluorescein (blue)], core sequence, forward (F) and reverse (R) primer sequences, repeat, repeat range and amplification range are given for each locus. N_w, N_a, H_O and H_E and *P* value represent number of individuals amplified successfully, number of alleles, observed heterozygosity, expected heterozygosity identified from 40 assayed individuals and *P* values of the test for deviation from Hardy–Weinberg expectations. Observed heterozygosities that are different from ($P < 0.05$) are listed in bold. Potential null alleles are signified by *.

were mixed with Applied Biosystems GeneScan 500 Rox size standard and then run on an ABI 3100 automated sequencer, and scored using the software GeneMapper 3.7 (Applied Biosystems). Tests for zygotic (Hardy–Weinberg) equilibrium and gametic disequilibrium were conducted in Arlequin (version 3.11) (Excoffier *et al*. 2005). A search for null alleles was conducted using Micro-Checker version 2.2.3 (van Oosterhout *et al*. 2006). We observed three to 14 alleles per locus, with an average of 7.3 alleles per locus. Expected heterozygosity values ranged from 0.311 to 0.891 (Table 1). All loci but one (SPU-A4) were in Hardy–Weinberg equilibrium (Table 1). An exact test for linkage disequilibrium between loci within the populations showed no locus pairs with significant *P* values after Bonferroni correction. Heterozygote deficit in only one population (Guadalupe Island) may either indicate the presence of null alleles or reflect the genetic isolation of such a remote island.

These 11 new loci are likely to be useful tools for ecological and fisheries investigations.

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