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PERMANENT GENETIC RESOURCES

Isolation and characterization of 12 microsatellites from the black surfperch, *Embiotoca jacksoni*, a reef fish that lacks a pelagic larval phase

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Abstract

A set of 12 microsatellite markers was developed from *Embiotoca jacksoni* genomic DNA and tested for polymorphism using 64 individuals from two populations. All loci were polymorphic with a number of alleles ranging from two to 19 with expected heterozygosities ranging from 0.17 to 0.89. There was no evidence of linkage disequilibrium and all loci were in Hardy–Weinberg equilibrium, except for four loci in one population. High numbers of private alleles were consistent with strong population structure, and very limited dispersal. Six microsatellite markers successfully cross amplified and were polymorphic in closely related species, *Embiotoca lateralis* and *Hyspurus caryi*.

Keywords: *Embiotoca jacksoni*, *Embiotoca lateralis*, *Hyspurus caryi*, microsatellites

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Surfperches (Family Embiotocidae) are coastal reef fishes that range from Baja California, Mexico, to Japan (Bernardi & Bucciarelli 1999). They are livebearers, with internal, delayed fertilization, an opportunity for sperm competition. Offspring are released when ready to recruit; thus, unlike most reef fishes, surfperches lack a pelagic larval phase (Bernardi 2000). These unique characteristics make embiotocids interesting at the ecological and evolutionary levels. In order to address parentage, connectivity, and population structure issues, we decided to develop microsatellite markers for one species, *Embiotoca jacksoni*, the black surfperch, where much ecological and evolutionary information is already available (e.g. Schmitt & Holbrook 1990; Bernardi 2000, 2005). Based on mitochondrial DNA sequences, previous work showed that black surfperch exhibit very limited realized dispersal, resulting in a strong genetic break in the Los Angeles region, separating northern and southern populations in two distinct clades (Bernardi 2000).

Genomic libraries enriched for microsatellite motifs were constructed by Genetic Identification Services (GIS, http://www.genetic-id-services.com). Libraries were built using a sample containing100 μg of genomic DNA extracted from muscle tissue from one individual *E. jacksoni* collected in Monterey, California following a standard phenol–chloroform procedure (Sambrook *et al*. 1989). Libraries were enriched for CA, CATC, TACA, and TAGA motifs. GIS sequenced 75 microsatellite-containing clones using universal M-13 primers. We tested 12 of these microsatellites, which were determined to have flanking sequences of length sufficient for primer design using Designer PCR version 1.03 (Research Genetics, Inc.). Amplification reactions were carried out in a total volume of 13 μL containing 11 μL of 1.1 PCR Mastermix (25 mM TAPS pH 9.5, 50 mM KCl, 2 mM $MgCl_2$, 200 μM each dNTP, *Taq* 5 U/μL, Thermo Scientific), 0.625 μL of both 20 μM primers and approximately 2 ng of DNA template. Using a fluorescently labelled forward primer and a cold reverse primer (Table 1), 35 PCR cycles were run in an Applied Biosystems GeneAmp PCR 9700 system, at denaturation, annealing and amplification temperatures of 94 °C, 52 °C, and 72 °C, for 30 s, 40 s, and 30 s, respectively.

Microsatellite amplifications 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 version 3.7 (Applied Biosystems). Sixty-four individuals from two populations, Monterey (38 individuals) and Catalina Island (26 individuals), taken as representatives of northern and southern clades (Bernardi 2000), were genotyped to estimate allelic diversity and calculate average observed and expected heterozygosities

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Table 1 Characterization of 12 polymorphic microsatellite loci for black surfperch, *Embiotoca jacksoni* from a sample of 64 individuals (38 from Monterey and 26 from Catalina Island). Columns correspond to: microsatellite name (Locus), (F) forward and (R) reverse primer sequence, repeat motif, number of alleles per locus (Na), number of private alleles (Pa), number of individuals successfully amplified (nw), amplification size of original clone (Amp. size), amplifications range (Amp. range), and (H_O) observed and (H_E) expected heterozygosities for the two tested populations. Asterisks (*) indicate loci departing from Hardy–Weinberg equilibrium. Three monomorphic loci were present in the Catalina population; thus, HWE calculations were nonapplicable, NA. GenBank Accession numbers are EU781555 to EU781566

								Monterey		Catalina Island	
Locus	Primer sequence (5′–3′)	Repeat motif	Na	Pa	nw	Amp. size	Amp. range	H_O	H_E	H_O	H_E
EJ_A2	F: 5′-AGCAAAGGTCAAAGGTCAA-3′ R: 5′-TTGTGGCTGTTGTTTATGG-3′	$(CA)_{20}$	9	5	57	235	233–249	0.441	0.726*	0.391	0.606
EJ_A5	F: 5′-AACCGCTGAGTAAGTAAACATC-3′ R: 5′-TCATCCCCATCATATTTATAGC-3′	$CA)_{30}$	19	15	55	275	271–325	0.892	0.853	0.889	0.852
EJ_A7	F: 5′-AATACCGTCGATGCTTTGTATC-3′ R: 5′-GCCTCTGATTATACGTCAGCTC-3′	$(CA)_{15}$	5	4	64	245	239–249	0.026	0.079*	0.077	0.076
EJ_A10	F: 5′-AACAAAAACTGCATCCAAGATG-3′ R: 5′-ACGAACTGTTCCATCCTCAAG-3′	$(CA)_{15}$	4	3	46	228	214–248	0.385	0.595*	NA	NA
EJ_A11	F: 5′-ACTTCCATGACAACAAAGTAGG-3′ R: 5′-CAAAATAAGCCAAGTGTGATG-3′	$(CA)_{24}$	10	9	62	283	263–293	0.594	0.646	0.440	0.431
EJ_A12	F: 5′-GAAAGAAGCTCAATGCAATCAC-3′ R: 5′-AGCAGCTCTCAGATCAGAGGTA-3′	$(CA)_{24}$	8	6	56	232	212–260	0.735	0.697	0.409	0.397
EJ_B1	F: 5′-ACTCGGACAGTAAAGCTGAGG-3′ R: 5′-AAAATGTCTCCTTGCAGGATC-3′	$(CATC)_{14}$	2	1	58	180	172–180	0.189	0.174	NA	NA
EJ_B3	F: 5′-CATTTTCCATCCATCCTTCTG-3′ R: 5′-CAGCACAAGCATCACATTAGC-3′	$(CATC)_{14}$	3	2	64	156	140–156	0.105	0.126	0.038	0.076
EJ_B5	F: 5′-CCACCTGGGGCTAAACTG-3′ R: 5′-CACGGCAGACAGAGCAAC-3′	$(CATC)_{15}$	3	1	62	112	108–112	0.250	0.438*	0.269	0.278
EJ_B8	F: 5′-GGTCGTATTTTGCAGTATGC-3′ R: 5′-AAGGATTCCCAACATCATG-3′	$(CATC)_{30}$	5	3	63	266	266–302	0.513	0.502	0.038	0.147
EJ_C3	F: 5′-CGTCAATGATACTCATGTGAAC-3′ R: 5′-ATGTCCCCTTGGGATTAA-3′	$(TAGA)_4(TACA)_8$	3	2	63	113	105–113	0.513	0.513	NA	NA
EJ_D2	F: 5′-CCTCCCTTTACCCATCTTTATC-3′ R: 5′-AAGGATATTGAGTCACCACAGG-3′	$(TAGA)_6$	13	10	60	283	283–347	0.611	0.564	0.833	0.839

at these 12 loci. All 12 primer pairs were polymorphic and successfully amplified most samples of *E. jacksoni* (see Table 1). Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were conducted in Arlequin (Excoffier *et al*. 2005). We observed two to 19 alleles per locus, and expected heterozygosity values ranged from 0.17 to 0.89. No significant LD was observed for any pair of loci ($P > 0.05$ for all comparisons). All polymorphic loci were in HWE in the Catalina Island population. A significant deviation from HWE was observed for four loci with heterozygote deficiency for the Monterey population (Table 1). Micro-Checker (Van Oosterhout *et al*. 2006) analysis suggested that the presence of null alleles was not responsible for the observed heterozygote deficit. Instead, it is likely that the unusual mode of dispersal and strong population structure in that species is responsible for this result. Indeed, the large number of private alleles (72%, Table 1) that characterize the two populations is consistent with the results obtained with mitochondrial DNA sequences (Bernardi 2000, 2005). A preliminary test of cross-reaction was performed on a limited number of loci and species. A subset of the developed primers were tested for cross-species amplification on two closely related embiotocid species (*Embiotoca lateralis*, 24 individuals, and *Hypsurus caryi*, four individuals). All tested primer sets amplified all samples and were polymorphic. Six primers sets were tested for *E. lateralis*, EJ_A2, EJ_A11, EJ_A5, EJ_A10, EJ_A12, EJ_B1, resulting in 9, 18, 12, 2, 3, and 5 alleles, respectively. Three primer sets were tested for *Hypsurus caryi*, EJ_A2, EJ_A10, EJ_A12, resulting in 6, 5, and 5 alleles, respectively. While this is a very restricted samples size, these data indicate the likely ability of these microsatellite markers to be used in closely related species. Our results therefore suggest that this new set of microsatellite markers will prove useful in parentage, population structure, and connectivity analyses.

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