

SHORT PAPERS AND NOTES

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MICROSATELLITE MARKERS FOR COBIA, *RACHYCENTRON CANADUM*.—Polymerase chain reaction (PCR) primers are reported for 35 nuclear-encoded microsatellites developed from a genomic library of cobia (*Rachycentron canadum*). All 35 microsatellites were tested for reproducibility and polymorphism using 24 cobia sampled offshore of Ocean Springs, MS. Thirty-three of the microsatellites were found to be polymorphic; genotypes at seven of these differed significantly from Hardy-Weinberg (HW) expectations, possibly because of the presence of null alleles. Levels of allele and gene diversity (expected heterozygosity) were lower on average than values reported previously for other marine fishes. The 26 microsatellites whose genotypes were in HW equilibrium should provide useful tools for future studies of cobia relating to both stock assessment and aquaculture.

Cobia is a migratory coastal pelagic fish distributed in tropical and subtropical warm waters worldwide except for the eastern Pacific (Shaffer and Nakamura, 1989). The species constitutes an important recreational fishery in the Gulf of Mexico (Brown-Peterson et al., 2001) and is caught incidentally in the commercial fishery (Shaffer and Nakamura, 1989). Interest in cobia aquaculture in the United States has spiked recently because of successes in captive spawning and larval rearing (Dodd, 2001), and it has been suggested (Bridger and Costa-Pierce, 2002) that cobia might be an ideal species for offshore cage culture.

In this note, we report optimized PCR primers for 15 nuclear-encoded microsatellites developed from a cobia genomic library. Briefly, microsatellites are short stretches of nuclear DNA composed primarily of di-, tri-, and tetranucleotide repeats inherited in a codominant (Mendelian) fashion and distributed throughout euchromatic regions of chromosomes (Weber and May, 1989; Weber, 1990; Wright and Bentzen, 1994). Microsatellites also accumulate mutations fairly rapidly (Shug et al., 1998), making them ideal genetic markers for a variety of applications ranging from stock structure analysis of “wild” populations (Gold and Turner, 2002; Zatzoff et al., 2004) to parentage assignment and pedigree reconstruction in domesticated populations (Wilson and Ferguson, 2002; Jones and Arden, 2003). In-

cluded in this note are summary data for 20 other microsatellites for cobia developed in our laboratory by Pruett et al. (2005). The summary data for all 35 microsatellites include number and size of alleles detected, observed and expected heterozygosity, and results of tests of conformity to Hardy-Weinberg equilibrium expectations at each microsatellite. The summary data are published here to allow convenient access to all PCR primers and other data.

Details regarding genomic library construction, ligation of size-selected (500–2,000 base pairs) fragments into cloning vectors and transformation into competent *Escherichia coli* cells can be found in Pruett et al. (2005). A total of 19,200 clones were hybridized with cocktails of oligonucleotide probes, and 164 positive clones were sequenced. A total of 54 clones containing microsatellite motifs were identified; 45 primer pairs were designed from sequences flanking the microsatellites with the programs Amplify 1.2 (Engels, 1993) and Net-primer (<http://www.premierbiosoft.com/netprimer>). Optimization of PCR protocols was carried out on DNA from eight individuals. PCR amplifications were performed in 10- μ l reaction volumes, consisting of 1 μ l (~25 ng) of DNA, 1 μ l of 10 \times reaction buffer (500 mM KCl, 100 mM Tris, 10% Triton-X 100), 0.1 U of *Taq* DNA polymerase (GibcoBRL), 0.5 μ M of each primer, 200 μ M of each dNTP, and 1 mM MgCl₂. PCR conditions consisted of an initial denaturation of 94 C for 3 min, followed by 30 cycles of denaturation at 94 C for 30 sec, annealing at optimized temperature (Table 1) for 45 sec, extension at 72 C for 1 min, and a final extension at 72 C for 10 min.

The primer-pair sequences (forward on top, reverse on bottom), microsatellite motifs (repeat sequence), size of cloned alleles, and optimized annealing temperatures (ATs) are given in Table 1. The suite of 35 microsatellites includes 26 di-, one tri-, and four tetranucleotide repeat motifs; four of the microsatellites contain complex repeats (i.e., a combination of different repeat motifs). Genotypes for all 35 microsatellites were acquired from 24 cobia sampled offshore of Ocean Springs, MS. The number of assayed individuals (N), the number of alleles (A_N), and the range in size of detected alleles for each microsatellite also are given in Table 1. Thirty-three of the microsatellites were found to be polymorphic; the av-

TABLE 1. Summary data for 35 microsatellites developed from a cobia (*Rachycentron canadum*) genomic library. PCR primer sequences are forward (top) and reverse (bottom). Primers *Rca* 1B-E08A and *Rca* 1B-E08B were developed from a single clone. Sequences of clones are listed under GenBank accession numbers AY721664–AY721682 and AY850008–AY850022. Significant deviations from Hardy–Weinberg expected proportions are in bold.

Microsatellite	PCR primer sequences (5' → 3')	Repeat sequence of cloned allele	Size of cloned allele (base pairs)	AT	N	A _N	Range in allele size (base pairs)	H ₀ /H _E	P _{HW}
<i>Rca</i> 1B-A10	GCAGCCAAATGCTAACAGCC CATGTAGTCAAGCGAGCCAGG	(GTT) ₆	180	60	24	6	169–187	0.417/0.723	0.000
<i>Rca</i> 1B-C06	CCAGCATATCTCCTCTCAAGA GGTTGAACTTAAGTACAGCTCCT	(GATA) ₂₉	346	50	23	13	340–404	0.870/0.904	0.370
<i>Rca</i> 1B-D09	CAGCTGCTTAGCCCTATCA GAAGGATGGACCACCTTGTGAC	(GT) ₉ (CTGT) ₂ (CT) ₂ (GT) ₂	167	60	23	1	168	0.000/0.000	1.000
<i>Rca</i> 1B-D10	GCAACTGCCTCCACCAATCA CATGTGCATCGAAAGACAGAGA	(CTAT) ₁₅	191	50	24	17	143–223	1.000/0.943	0.597
<i>Rca</i> 1B-E02	GTGTTGCAGCCAAATGCTA CTCCCTAGTGCCACTACAGCTC	(CT) ₁₈	308	60	24	7	297–313	0.667/0.598	0.503
<i>Rca</i> 1B-E06	GGATCAGTGTGTCAGCCA CCCTAGTGCCTACAGCTCCCT	(TC) ₁₈	314	45	24	8	305–327	0.625/0.695	0.000
<i>Rca</i> 1B-E08A	CATATCAAGTCAATACAGACC CCAGGAAATPAGCAGACTTCTC	(CA) ₃ CA(CA) ₅ A(CA) ₁₆	227	55	24	5	181–225	0.458/0.582	0.028
<i>Rca</i> 1B-E08B	GCAGTTGATCTGATGCTACAC CTAATGCCAGCTCATATATGTC	(CA) ₈ GA(CA) ₃	122	60	24	2	121–123	0.458/0.510	0.692
<i>Rca</i> 1B-F06	CAAGCAAAATGCGTGCCCGA CGTTAGCAAACACAGGAGCTTG	(CTAT) ₁₅	268	55	24	11	260–300	0.542/0.796	0.000
<i>Rca</i> 1B-F07	GGAAATCTGGTGGTGGTCAAT CTGTGGCTGAAAGCGTGTGT	(GACA) ₆ (CA) ₁₂	140	55	24	3	131–139	0.083/0.082	1.000
<i>Rca</i> 1B-G10	GGAACTCTATAACAGCATGTC GTAGACAGAGCAACACATGAG	(CT) ₅ TT(CT) ₄	154	55	23	2	153–155	0.043/0.043	1.000
<i>Rca</i> 1-H09	CATGTTATTTCCAACTCATGG GTGATCCGCATACTTTTTCAG	(GATA) ₃₁	220	48	23	12	176–224	0.957/0.910	0.343
<i>Rca</i> 1-A04	CAGGCATGCCTACTTTAACC GCTGTTGATGTGGCGAAGCAAC	(CA) ₉ (CACT) ₄	202	60	24	6	196–206	0.625/0.722	0.107
<i>Rca</i> 1-A08	GGATCATAAAGGGATTGTGCTA CCTCAGCCATATCATCAT	(GT) ₁₃ GCAT(GT) ₅	289	48	24	8	287–321	0.208/0.575	0.000
<i>Rca</i> 1-A11	CTACAGTGGTGTCCCTGTTAG CAGTACATAGAGAAAACAGGAGG	(GT) ₂₄	187	55	24	15	167–201	0.792/0.889	0.265
<i>Rca</i> 1-B12	GCTTCAGGCAAGTGAGACC GGGAGGTAATATGTCCTGT	(AC) ₉	181	55	24	9	176–196	0.500/0.808	0.000
<i>Rca</i> 1-C04	GACATCAAGTGGCACTTTC CACTAAACTTGTTCCTCCTG	(GT) ₁₇	219	48	24	10	223–253	0.667/0.641	0.185

TABLE 1. Continued.

Microsatellite	PCR primer sequences (5' → 3')	Repeat sequence of cloned allele	Size of cloned allele (base pairs)	AT	N	A _n	Range in allele size (base pairs)	H _o /H _e	P _{HW}
<i>Rca</i> 1-D04	GCTGAACCTTGCGCGCT GGACTGAACCTCCCTATCCTC	(TG) ₉ AC(TG) ₅	127	60	24	3	125-129	0.667/0.551	0.733
<i>Rca</i> 1-D07	CCATGGCTACAATCTGGTTCAATC CGAATGCTGTGGAGAACAGG	(GT) ₉ TTT(GT) ₃	157	60	23	4	154-162	0.130/0.128	1.000
<i>Rca</i> 1-D08	GCTTGACTCCAGCTCAAC CACAGGACGAGCTCCA	(CA) ₁₀	172	55	23	4	172-178	0.261/0.274	0.074
<i>Rca</i> 1-D11	CGTAACACCTTTGGAAGACATC CTCCAATTGAGGCTACTAGTG	(GT) ₈	208	55	24	4	204-212	0.333/0.295	1.000
<i>Rca</i> 1-E04	CCAAGAACAGCGGGCAAC GCCACCATTGTGTGGGTGA	(CA) ₈ bp(CA) ₅	220	55	23	4	215-237	0.391/0.336	1.000
<i>Rca</i> 1-E05	GCAGTCGAGACTGACTGAACGA CGTGGAGCTGCTCTGCAGGA	(CA) ₃₀ (CGCA) ₄ (CA) ₇ (CGCA) ₄	248	55	24	8	241-259	0.542/0.768	0.000
<i>Rca</i> 1-E06	GGCACCAATCACTACTACTG TGTTGAGGTCATCACTGTC	(CA) ₃₉	180	48	24	9	144-186	0.458/0.826	0.000
<i>Rca</i> 1-E11	GTCCAGCTCCAGCCCAAC GACACTGGCTCGGTGAGCA	(CA) ₁₂	173	55	23	7	167-181	0.783/0.757	0.236
<i>Rca</i> 1-F01	GCTCAFTTCTACTAAGTGTGTAGC CCATGAATCTACATTCACCTGCCA	(TG) ₁₂	198	60	24	2	201-205	0.125/0.120	1.000
<i>Rca</i> 1-F07	CATCGGGTTGAGTTGTACT CGTTGCCTGTCAATCTGTGCT	(CA) ₆ CG(CA) ₃	235	60	23	1	235	0.000/0.000	1.000
<i>Rca</i> 1-F10	CCGTTCTGTACAGACGTGAAC GCCCTGTGCTGTTCTCTGCA	(CA) ₂ CG(CA) ₁₂ CG(CA) ₄	287	55	23	5	287-297	0.261/0.423	0.004
<i>Rca</i> 1-F11	GTTGCCATGGCGACCGAGA GCCCTATGCTCGTTCATC	(GA) ₈ AA(GA) ₅	122	55	24	2	119-121	0.000/0.082	0.022
<i>Rca</i> 1-G02	GGACCAATGTGAACATCATGCT CCAGACATGGACTGGTACACCT	(GT) ₁₄	238	60	23	2	240-244	0.043/0.043	1.000
<i>Rca</i> 1-G05	GGGTGCTGCTGGCTGTAA GCATCTGTCTCTGGTGAGAGTC	(GT) ₁₇	280	60	24	5	275-283	0.667/0.651	0.185
<i>Rca</i> 1-H01	GTCCCAAGGAATAGCGAAG CCTCCAGCAGACAGCAGA	(CA) ₃₇	298	48	23	12	275-311	0.826/0.885	0.129
<i>Rca</i> 1-H04A	GGGCTTTACGAAAAGATAGCTGA GAGACTACATGGCAGAGGT	(GT) ₁₈	161	60	24	3	156-162	0.667/0.550	0.269
<i>Rca</i> 1-H08	GACCACTCCTTTGAGGTCCT GCACCCACTGCACAACAC	(GT) ₃₀	278	60	24	9	273-299	0.708/0.696	0.984
<i>Rca</i> 1-H10	GCTGTGCATACTCACACTGCT	(CA) ₁₆	121	60	24	8	119-139	0.583/0.777	0.145

erage number of alleles per polymorphic microsatellites was 7.1 (range 2–17). Estimates of observed (H_o) and expected (H_e) heterozygosity were computed with the Genetic Data Analysis (GDA) program (Lewis and Zaykin, 2001) and are given in Table 1. For the polymorphic microsatellites, average observed heterozygosity was 0.496 (range 0.000–1.000), whereas the average expected heterozygosity was 0.563 (range 0.043–0.943). The average number of alleles and average expected heterozygosity (also called gene diversity) per microsatellite are lower than averages reported previously by DeWoody and Avise (2000) for several species of marine fishes. Probabilities of departure from Hardy-Weinberg equilibrium expectations (P_{HW}) were computed by exact tests, as implemented in GDA (Lewis and Zaykin, 2001) and are given in Table 1. Genotypes at seven of the microsatellites differed significantly from Hardy-Weinberg equilibrium expectations following (sequential) Bonferroni correction for multiple tests performed simultaneously (Rice, 1989). Results of analysis by Microchecker (Van Oosterhout et al., 2004) indicated that six of these seven microsatellites (all but *Rca* 1B-E06) had a general excess of homozygotes for most allele size classes, suggesting the presence of null alleles. The 26 microsatellites whose genotypes were in HW equilibrium should prove extremely useful in future studies of cobia relating to both stock assessment and aquaculture. The use of microsatellites as selectively neutral genetic markers to assess geographic boundaries and genetic diversity of “wild” stocks is well reviewed in Wright and Bentzen (1994) and Carvalho and Hauser (1995); the use of microsatellites in aquaculture includes parentage assignment, pedigree reconstruction, mapping of quantitative trait loci, and marker-assisted selection and is well reviewed in Liu and Cordes (2004).

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