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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; Zatcoff et al., 2004) to parentage assignment and pedigree reconstruction in domesticated populations (Wilson and Ferguson, 2002; Jones and Arden, 2003). Included 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 Netprimer (http://www.premierbiosoft.com/netprimer). Optimization of PCR protocols was carried out on DNA from eight individuals. PCR amplifications were performed in 10-μl reaction volumes, consisting of 1 μl (~25 ng) of DNA, 1 μl of 10× reaction buffer (500 mM KCl, 100 mM Tris, 10% Triton-X 100), 0.1 U of *Taq* DNA polymerase (GibcoBRL), 0.5 μM of each primer, 200 μM of each dNTP, and 1 mM $MgCl_2$. 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_O/H_E	P_{HW}
Rca 1B-A10	GCAGCCCAATGCTAACAAGCC CATGTAGTCAAGCGAGCCACG	$(GTT)_6$	180	60	24	6	169–187	0.417/0.723	**0.000**
Rca 1B-C06	CCAGCATATCTCCTCTCAAGA GGCTTGAACTTAACTACAGCTCCT	$(GATA)_{29}$	346	50	23	13	340–404	0.870/0.904	0.370
Rca 1B-D09	CAGCCTGCTTAGCCTATCA GAAGGATGGACCACTTGTGAC	$(GT)_9(CTGT)_2(CT)_2(GT)_2$	167	60	23	1	168	0.000/0.000	1.000
Rca 1B-D10	GCAACTGCCTCCACCAATCA CATGTGCATCGAAAGACAGAGA	$(CTAT)_{15}$	191	50	24	17	143–223	1.000/0.943	0.597
Rca 1B-E02	GTGTTGCAGCCAAATGCTA CTCCCTAGTGCCACTACAGCTC	$(CT)_{18}$	308	60	24	7	297–313	0.667/0.598	0.503
Rca 1B-E06	GGATCAGTGTGTTGCAGCCA CCCTAGTGCCACTACAGCTCCCT	$(TC)_{18}$	314	45	24	8	305–327	0.625/0.695	**0.000**
Rca 1B-E08A	CATATCAAGTCAATATCACAGACC CCACGGAATAGCAGACTTTCTC	$(CA)_3GA(CA)_5A(CA)_{16}$	227	55	24	5	181–225	0.458/0.582	0.028
Rca 1B-E08B	GCAGTTGATTCTGATTGCTACAC CTAATGCCAGCTCATTATGTCC	$(CA)_8GA(CA)_3$	122	60	24	2	121–123	0.458/0.510	0.692
Rca 1B-F06	CAAGCAAATGCGTGGCCGA CGTTAGCAACCACACGAGCTTG	$(CTAT)_{15}$	268	55	24	11	260–300	0.542/0.796	**0.000**
Rca 1B-F07	GGAATCTGGTGGTGAGTCAT CTGTGGCTGAAGCGTGTGTT	$(GACA)_6(CA)_{12}$	140	55	24	3	131–139	0.083/0.082	1.000
Rca 1B-G10	GGAAACTCTATAACAGCATGTC GTAGACAGAGCAACACATGAG	$(CT)_5TT(CT)_4$	154	55	23	2	153–155	0.043/0.043	1.000
Rca 1-H09	CATGTTATTCTCCAACTCATGG GTGTATCCGCATACTTTCAG	$(GATA)_{31}$	220	48	23	12	176–224	0.957/0.910	0.343
Rca 1-A04	CACGCACATGCACTACTTTAACC GCTGTTGATGTGGCGAAGCAAC	$(CA)_9(CACT)_4$	202	60	24	6	196–206	0.625/0.722	0.107
Rca 1-A08	GGATCATAAGGGATTGTGCTA CCTCGAGCCATATCATCAT	$(GT)_{13}GCAT(GT)_5$	289	48	24	8	287–321	0.208/0.575	**0.000**
Rca 1-A11	CTACAGTGGTGTTCCCTGTTAG CAGTACATAGAGAAACAGGAGG	$(GT)_{24}$	187	55	24	15	167–201	0.792/0.889	0.265
Rca 1-B12	GCTTCAGGCAAGTGAGACC GGGAGGTAATTATGTCCTGT	$(AC)_9$	181	55	24	9	176–196	0.500/0.808	**0.000**
Rca 1-C04	GACATCAAGTGGCACTTTG CACTAAACTTGTTCCTCCTG	$(GT)_{17}$	219	48	24	10	223–253	0.667/0.641	0.185

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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}
Rca 1-D04	GCTGAACTTGTCGCCGCT GGACTGAACCTCCCTATCCTC	$(TG)_9AC(TG)_5$	127	60	24	3	125–129	0.667/0.551	0.733
Rca 1-D07	CCATGGCTACAATCTGGTTCATC CGAATGCTGTGGAGAACAGG	$(GT)_9TTT(GT)_3$	157	60	23	4	154–162	0.130/0.128	1.000
Rca 1-D08	GCTTGACTCCAGCTCAAAC CACAAGGACGAGCCTCCA	$(CA)_{10}$	172	55	23	4	172–178	0.261/0.274	0.074
Rca 1-D11	CGTAACACCTTTGGAAGACATC CTCCATTGAGGCTGACTAGTG	$(GT)_8$	208	55	24	4	204–212	0.333/0.295	1.000
Rca 1-E04	CCAAGAACAGGCGGGCAAC GCCACCATTGTGTGTGGGTGA	$(CA)_88bp(CA)_5$	220	55	23	4	215–237	0.391/0.336	1.000
Rca 1-E05	GCAGTCGAGACGTGACTGAACGA CGTGGAGCTGCTCTGCAGGA	$(CA)_{20}(CGCA)_4(CA)_7(CGCA)_4$	248	55	24	8	241–259	0.542/0.768	**0.000**
Rca 1-E06	GGCACCAATCACTCACTACTG TGTTGAGGTCTATCAGTGCC	$(CA)_{39}$	180	48	24	9	144–186	0.458/0.826	**0.000**
Rca 1-E11	GTCCCAGCTCCAGCCCAAAC GACACTGGCTGCGTGAGCA	$(CA)_{12}$	173	55	23	7	167–181	0.783/0.757	0.236
Rca 1-F01	GCTCATTTCACTAAGTGTGTTGTAGC CCATGAATCTACATTCACCTGCCA	$(TG)_{12}$	198	60	24	2	201–205	0.125/0.120	1.000
Rca 1-F07	GCATCGGGTTGAGTTGTACT CGTTGCCTGTCAATCTGTGCT	$(CA)_6CG(CA)_3$	235	60	23	1	235	0.000/0.000	1.000
Rca 1-F10	CCGTTCTGTACAGACGTGAAC GCCTGTTGCTGTTTCCTGTCA	$(CA)_2CG(CA)_{12}CG(CA)_4$	287	55	23	5	287–297	0.261/0.423	0.004
Rca 1-F11	GTTGCCATGGCGACCGAGA GCCCCTATGTCTCGTTCCATC	$(GA)_8AA(GA)_5$	122	55	24	2	119–121	0.000/0.082	0.022
Rca 1-G02	GGGACCATGTGAACTCATGCT CCAGACATGGACTGGTACACCT	$(GT)_{14}$	238	60	23	2	240–244	0.043/0.043	1.000
Rca 1-G05	GGGCTGTCTGCTGGCTGTAA GCATCTGTGTCCTGGTGAGAGTC	$(GT)_{17}$	280	60	24	5	275–283	0.667/0.651	0.185
Rca 1-H01	GTCCCAAGGGAATAGCGAAG CCTCCAGACCAGACAGCAGA	$(CA)_{37}$	298	48	23	12	275–311	0.826/0.885	0.129
Rca 1-H04A	GGGAGCCATGTGGTACAGACT GGGCTTTACGAAAGATAGCTGA	$(GT)_{18}$	161	60	24	3	156–162	0.667/0.550	0.269
Rca 1-H08	GAGACCTACATGGCAGAAGGT GACCACTCCTTTGAGGTCTCT	$(GT)_{30}$	278	60	24	9	273–299	0.708/0.696	0.984
Rca 1-H10	GCACCGCACTGCACAACAC 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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