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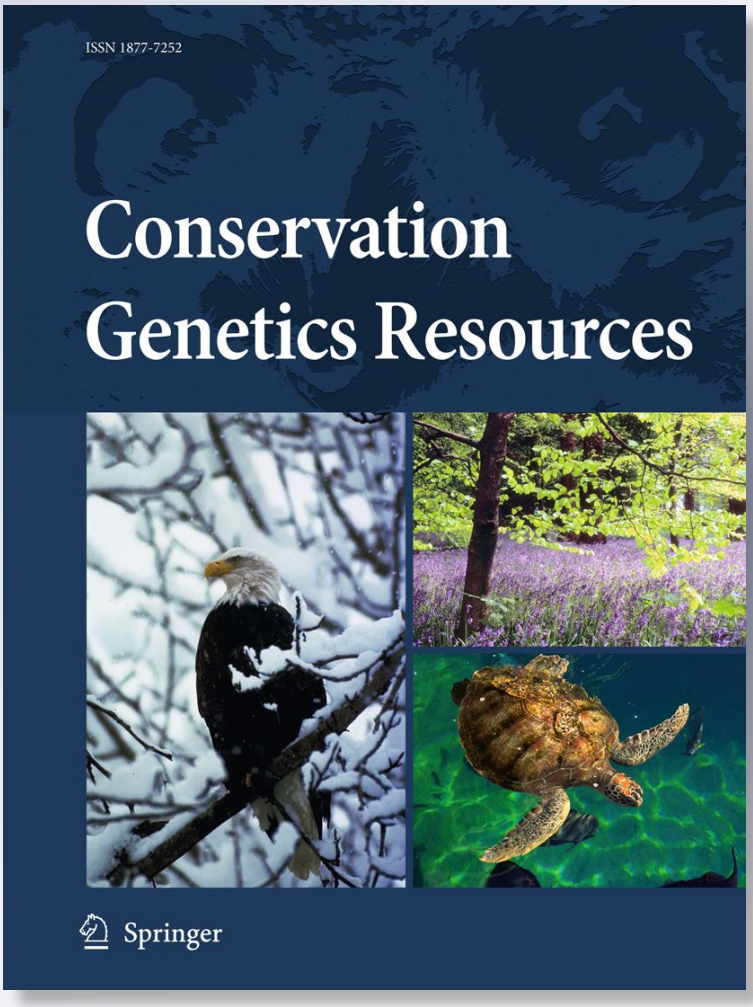

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TECHNICAL NOTE

Isolation and characterization of microsatellite markers for the dusky smoothhound shark, *Mustelus canis*

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Abstract A total of 32 nuclear-encoded microsatellites, including 15 polymorphic and 13 monomorphic microsatellites, isolated from an enriched genomic library of the triakid shark, *Mustelus canis* (dusky smoothhound), and four microsatellites previously isolated from two other triakid sharks were characterized in 91 individuals of *M. canis*. Polymorphic repeat motifs included 12 dinucleotide, two trinucleotide and one pentanucleotide repeat(s). *Mustelus canis* supports both commercial and recreational fisheries throughout its range in the western Atlantic Ocean from Maine (USA) to southern Argentina. The microsatellites characterized will be useful in studies of population structure of *M. canis* and other triakid sharks.

Keywords Microsatellites · Dusky smoothhound shark · *Mustelus canis* · Triakidae

The dusky smoothhound shark, *Mustelus canis*, is a small demersal shark found in temperate waters along the continental shelf of the western Atlantic Ocean from Canada to southern Argentina (Compagno et al. 2005). The species is currently listed as 'Near-Threatened' by the IUCN red-list (Conrath 2005) and little is known about its population structure. Bigelow and Schroeder (1948) hypothesized that there are several distinct stocks of *M. canis* throughout its range, suggesting that an assessment of stock structure for the species will prove important for future conservation of dusky smoothhound resources. Polymorphic nuclear-encoded microsatellites have proven useful for detecting population structure in elasmobranchs on both large and small scales (Plank et al. 2010; Portnoy et al. 2010). Here, we describe development and characterization of 28 microsatellites (15 polymorphic) from an enriched genomic library of *M. canis,* as well as characterization in *M. canis* of four microsatellites developed for the triakid sharks *Galeorhinus galeus* (*Gg*3, *Gg*16; Chabot and Nigenda 2011) and *Mustelus antarcticus* (*Ma*FYP, *Ma*WS1; Boomer and Stow 2010), respectively.

Generation of the enriched genomic library followed procedures outlined in Renshaw et al. (2010). Two separate hybridization reactions were performed; one with 50 pmol of 3′-biotin modified $(CA)_{13}$ and the other with $(CAT)_8$ and $(GAT)_8$ oligonucleotides. Hybridization mixtures were heated to 95°C for 10 min and then kept at 58°C [$(CA)_{13}$ hybridization] and 47°C [$(CAT)_8$ and $(GAT)_8$ hybridization] for 1.25 h. Enriched genomic fragments were ligated into the pCR®2.1-TOPO® vector (Invitrogen) and transformed into *Escherichia coli* (One Shot® TOP10 Chemically Competent Cells, Invitrogen). Positive (white) clones were sent to University of Florida's Interdisciplinary Center for Biotechnology Research (http://www.biotech.ufl.edu/) for sequencing with M13 primers. Sequences were edited and vectors trimmed with SEQUENCHER 4.1 (Gene Codes). Primer pairs were developed using Primer3plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi).

Initial PCR reactions followed Boutin-Ganache et al. (2001) and employed a forward primer with an attached 21-bp tail sequence (5′-GCCTCGTTTATCAGATGTGGA-3′) labeled with either 6-FAM, HEX or NED (Dye Set D, Applied Biosystems) and an unlabeled reverse primer; forward and reverse primers were purchased from Integrated DNA technologies (IDT). Primer pairs yielding clean amplifications were run on 24 individuals to identify polymorphic microsatellites. Nineteen of the microsatellites (15 from the

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Table 1 Summary data for 32 microsatellites characterized in the dusky smoothhound shark, *Mustelus canis*

Microsat	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone size[d]	T_A[e]	N/N_A[f]	Range[g]	H_E[h]	H_O[i]	P_{HW}[j]
Polymorphic microsatellites										
*Mca*31	GGCAGATCAGTTGAGGAAGG AATGGGGAGACTTCTCTTTGC	JN083992	$(ATC)_4$	237	55	91/4	229–247	0.399	0.407	0.048
*Mca*33	CATTTGAACCCCGACAGAAC TCCAAGTAAGGATGAGTGACACC	JN083993	$(ATC)_5$	201	58	91/2	197–200	0.022	0.022	1.000
*Mca*40	AGCTCTGTCCAATCCAAGCT CAATTTATTATTGTTCAGAT	JN083994	$(AC)_5$	170	58	88/2	162–170	0.488	0.443	0.393
*Mca*44	TTTCCGCTGTATCACACATACAC GCATCTATATGTCTGCGTGTGTC	JN083995	$(AC)_{11}$	179	58	90/10	169–187	0.772	0.800	0.048
*Mca*B5	TAATCGACACGCAGTCATCG AAGCTCCAATTCTCACTGTGC	JN083996	$(GT)_{11}$	196	52	91/5	192–212	0.626	0.593	0.851
*Mca*B6	AGGATAAATACACGCACACAGG TTTTTGTTTTGCAATCTCACG	JN083997	$(CA)_{10}$	248	52°	91/7	238–254	0.186	0.165	0.017
*Mca*B22	TCCTCTCCAGGACAAACACAC TCCCACCTGCCATAGTAATTG	JN083999	$(AC)_{18}$	168	62	90/14	139–173	0.859	0.744	0.004
*Mca*B26	ACTGTGGCACTGCATTCTGC TGCATTTCAAAACCACTGGA	JN084000	$(AAATC)_5$	230	55	91/3	225–235	0.266	0.286	1.000
*Mca*B28	GGAGGAGCTAAGGGAAAAGC TCCTCAAGCTTCCAGAACACT	JN084001	$(TC)_8$	150	62	90/3	144–154	0.055	0.056	1.000
*Mca*B33	TCTCCTAATGGAACGTGTGC GGTATGCGTATGGGTGTCG	JN084002	$(CA)_5$	155	55	91/5	154–166	0.522	0.593	0.566
*Mca*B35	AGTGCGTGCCAGTGTATGAG GTTCTGCATGGGACGTGAC	JN084003	$(TG)_8$	210	58	91/4	186–212	0.420	0.352	0.103
*Mca*B36	TTGGCTCGTTAAGGGTATGTG TTCTTTATCCCGTCGATTCC	JN084004	$(GT)_{10}$	155	62	91/3	150–164	0.531	0.451	0.002
*Mca*B37	TCTGCCTCTGTGTCTCATCC TTTCCATTTCCGACATAGGG	JN084005	$(GT)_5$	236	55	91/4	239–255	0.477	0.407	0.174
*Mca*B40	TGGCATTCCATTTGCTGATA TGTCAGCACAGGAGGGTGTA	JN084006	$(CA)_6$	170	64	90/5	166–171	0.507	0.511	0.199
*Mca*B41	TGTGCTATCACACGGAGTGG CTCACCCCCTCTCTTTCTCC	JN084007	$(TG)_5TTT(GT)_2(GA)_8$	207	58	90/2	205–209	0.427	0.389	0.443
*Gg*3	CCGTGACTGAAAGCAGCC CCCTCAACCATGGCAAGTG	N/A	$(GATT)_N$	*	58	91/2	241–249	0.022	0.022	1.000
*Gg*16	AGTGTGGTCTCACCAATGC TGGAAGGGTAAGGAAATTGGC	N/A	$(GA)_N$	*	N/A	90/4	184–190	0.518	0.544	0.851
*Ma*FYP	TGGTTGCCGATACAGCAGG CAAGCGCATGCACACTCAC	N/A	$(GT)_{11}(GT)_4$	*	58	91/8	238–260	0.760	0.725	0.473
*Ma*WS1	CGTAGCCAACCATTCCTGTT GAGCGTAGGGAGGTCAAGG	N/A	$(GT)_{15}$	*	60	91/2	181–191	0.011	0.011	1.000
Monomorphic microsatellites										
*Mca*24	AAACTGCTGGCCTTGTCAAC AATCAGCACAAAGGGAGTGG	JN129144	$(GT)_5$	154	N/A	87/1	176	N/A	N/A	N/A
*Mca*25	ACACACTTTCACGCACAAGC TCGCTCAAGTGAGACCAGAG	JN129145	$(CA)_3(CT)_5$	240	N/A	85/1	260	N/A	N/A	N/A
*Mca*32	TCATTAAACCCGGACTTTGC CGACGAGCCTGATATGTGTG	JN129146	$(GA)_6$	237	N/A	90/1	258	N/A	N/A	N/A
*Mca*38	AATCAGCACAAAGGGAGTGG AAACTGCTGGCCTTGTCAAC	JN129147	$(AC)_5$	154	N/A	88/1	175	N/A	N/A	N/A

Table 1 continued

Microsat	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone size[d]	T_A[e]	N/N_A[f]	Range[g]	H_E[h]	H_O[i]	P_{HW}[j]
*Mca*B4	TGTAAACAATCAGTGGCAAGC AAATTTGGAACGAGTGTCTGC	JN129148	$(CA)_7$	206	N/A	89/1	226	N/A	N/A	N/A
*Mca*B7	CCTCGATGACTAATGCAAAGC GTGGGGACATGTTTGTGTGC	JN129149	$(CA)_5$	283	N/A	75/1	304	N/A	N/A	N/A
*Mca*B16	AGGAGGATGCAGAGATTTGG ACTGATGCACGAGGACACC	JN129150	$(TG)_7$	196	N/A	88/1	208	N/A	N/A	N/A
*Mca*B20	CCTTCAGGAAGGCAAAACC TTGGGTTTTAATGGGGATAGC	JN129151	$(AG)_6$	104	N/A	87/1	124	N/A	N/A	N/A
*Mca*B21	CATGCCACGTGATAGTGAGG TACCCCTCTGGTTCAAATGC	JN129152	$(GA)_5$	169	N/A	85/1	190	N/A	N/A	N/A
*Mca*B24	CGGGACACCGGAATAGATTA GATCAGATCCCTCCGTACCA	JN129153	$(TG)_6$	243	N/A	77/1	255	N/A	N/A	N/A
*Mca*B27	ATCCAGTGGTTTTGAAATGC CCTCGTAGGTCTCGTC	JN129154	$(GT)_6$	166	N/A	86/1	189	N/A	N/A	N/A
*Mca*B29	ACAATGGACACAGCAAGAGC CCCCTCTCAGTCTCACTCTCC	JN129155	$(AG)_7$	102	N/A	85/1	135	N/A	N/A	N/A
*Mca*B39	GGACAGGCAGCATCTGTGTA CCCAGGGGGATTAGGATATT	JN129156	$(CA)_{10}GAT(AC)_8$	231	N/A	74/1	201	N/A	N/A	N/A

[a] Primer sequences are forward (top) and reverse (bottom)

[b] Genbank accession number

[c] Repeat indicates repeat motif

[d] Clone size is the size (in base pairs) of the allele in the sequenced clone

[e] Annealing temperature in °C

[f] N is number of individuals assayed; N_A is number of alleles detected

[g] Range refers to size in base pairs of alleles thus far uncovered

[h] H_E is expected heterozygosity

[i] H_O is observed heterozygosity

[j] P_{HW} represents probability of deviation from Hardy–Weinberg expectations

* Original clone size not given in Chabot and Nigenda (2011) or Boomer and Stow (2010)

M. canis library and four from two other triakids) were polymorphic. All 32 microsatellites were characterized on an additional 67 individuals of *M. canis*; for amplifications of all but one polymorphic microsatellite, the forward primer was directly labeled with either HEX or 6-FAM. The 21-bp-tail protocol of Boutin-Ganache et al. (2001) was used to characterize alleles at *Gg*16 and alleles at the 13 monomorphic microsatellites developed from the *M. canis* library. All individuals assayed were obtained in Delaware Bay, USA. Amplicons were electrophoresed on an ABI 377 automated sequencer with a 400HD [Rox] Size Standard (Applied Biosystems). Allele sizing and calling were performed using GENESCAN® version 3.1.2 and GENOTYPER® version 2.5 software (Applied Biosystems).

Genetic variability for each microsatellite marker was measured as number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity, as calculated in GDA (Lewis and Zaykin 2001). A Fisher's exact test, as implemented in GDA (Lewis and Zaykin 2001), was used to test for significant departures from expectations of Hardy–Weinberg equilibrium at each microsatellite. MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004) was utilized to check for the presence of null alleles, large-allele dropout, and/or stuttering at each microsatellite. Summary data for 32 microsatellites, 28 developed from the genomic library of *M. canis* and for four developed in the two other triakid sharks (Chabot and Nigenda 2011; Boomer and Stow 2010) are presented in Table 1. The number of alleles detected ranged from 2 (*Mca*33, *Mca*40, *Mca*B28, *Mca*B40, *Mca*B41, *Gg*3, *Ma*WS1) to 14 (*Mca*B22); expected heterozygosity ranged from 0.011 (*Ma*WS1) to 0.859 (*Mca*B22), while observed heterozygosity ranged from 0.011 (*Ma*WS1) to 0.798 (*Mca*44). Genotypes at *Mca*B36 deviated significantly from Hardy–Weinberg (HW) expectations following sequential Bonferroni correction (Rice 1989). The probability (*P*) that genotypes at *Mca*B22 did not fit HW expectation was close

to the Bonferroni-corrected significance value of 0.003; the corrected *P* value, however, was 0.068, suggesting that genotypes at *Mca*B22 are not necessarily out of HW equilibrium. Evidence of one or more null alleles at *Mca*B22 was suggested by analysis with MICROCHECKER. Single base-pair shifts in the dinucleotide microsatellite *Mca*B40 were detected in three individuals, but the alleles were easily scored. The microsatellites characterized here will prove useful for population genetic studies of *Mustelus canis* and potentially for other species in the family Triakidae.

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