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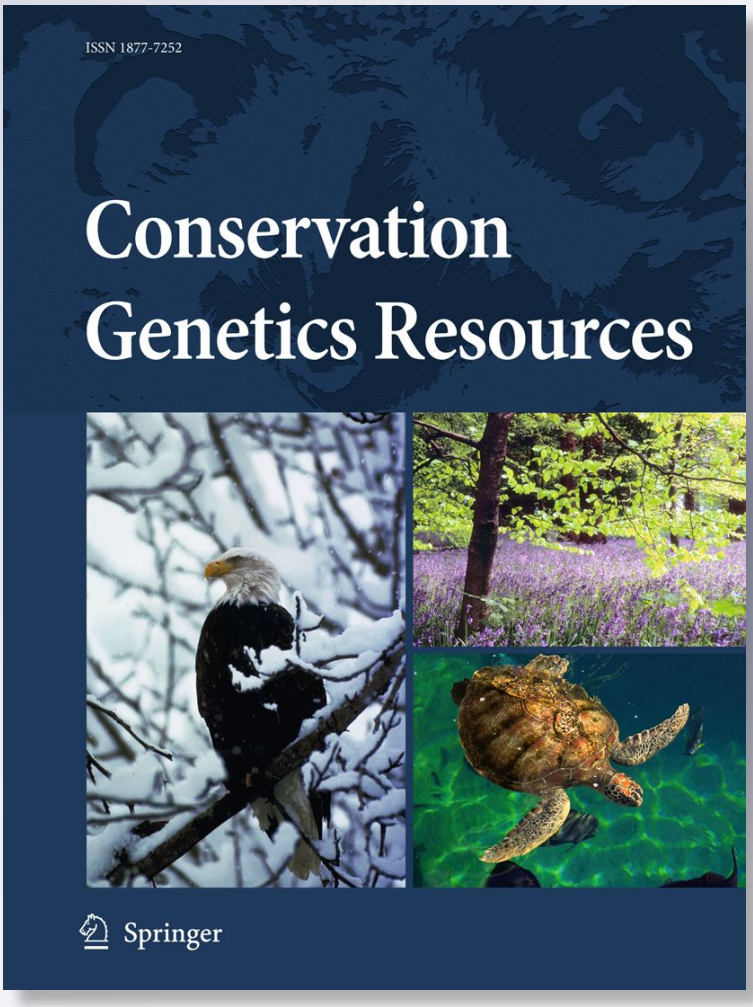

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TECHNICAL NOTE

Isolation and characterization of microsatellite markers for the blacknose shark, *Carcharhinus acronotus*

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Abstract The blacknose shark, *Carcharhinus acronotus*, is distributed in coastal waters of the western Atlantic Ocean from North Carolina to Brazil and is a component of both commercial and recreational fisheries throughout its range. In waters off of the United States, the species is managed as a component of the small coastal shark (SCS) complex. Concerns recently have arisen that blacknose sharks are overfished and experiencing overfishing. Here, we report polymerase-chain-reaction (PCR) primers for 37 polymorphic and 26 monomorphic microsatellites isolated from an enriched genomic library of *C. acronotus* DNA and characterized in 32 individuals. Polymorphic repeat motifs included 36 dinucleotides and one pentanucleotide. The characterized microsatellites will be useful in studies which involve conservation and management of blacknose shark resources.

Keywords Microsatellites · Blacknose shark · *Carcharhinus acronotus*

The blacknose shark, *Carcharhinus acronotus,* is a small, migratory coastal shark found in tropical and subtropical waters along the continental shelf of the western Atlantic Ocean from North Carolina (USA) to Brazil and including the Caribbean Sea (Compagno et al. 2005). The species is currently listed as 'Near-Threatened' by the IUCN Red-List (Morgan et al. 2008) and is caught in both directed fisheries and as by-catch. Although there is a paucity of information on the population structure of *C. acronotus*, differences in several life-history characteristics between blacknose sharks in the U.S. South Atlantic and the Gulf of Mexico are compatible with the hypothesis that there may be separate reproductive units in U.S. waters (Driggers et al. 2004; Sulikowski et al. 2007). An assessment of blacknose sharks in U.S. waters recommended managing the species as a single stock but stressed the need for a more rigorous assessment of population structure (NMFS 2007). Here, we describe the development and characterization of 63 microsatellites from an enriched genomic library of *C. acronotus* DNA. The microsatellites will be useful in determining if multiple stocks exist and in conservation and management of the species.

Generation of the enriched genomic library followed procedures outlined in Renshaw et al. (2010). A hybridization reaction was performed using 50 pmol of a 3′-biotin modified $(CA)_{13}$ oligonucleotide. The hybridization mixture was heated to 95°C for 10 min and then kept at 58°C 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′-GCCTCGTTTATCAGATGTG GA-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

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Table 1 Summary data for 63 microsatellites characterized in the blacknose shark, *Carcharhinus acronotus*

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										
*Cac*1	TATTTCCCGCAGTTGTACCC ACCGAACGGATCTGAGACTG	JN253437	$(CA)_{13}$	172	54	32/6	167–181	0.417	0.406	0.019
*Cac*3	ATCGACTCCATGCAGAATCC TGCCCAATGAACAAACAAAA	JN253438	$(CA)_{15}$	232	54	32/11	230–254	0.844	0.844	0.066
*Cac*8	GTGCAGATATACATACATCA CACACT GTATTGCTTGGTGCGAGGTT	JN253440	$(CA)_7$	119	54	31/3	94–116	0.650	0.742	0.678
*Cac*14	CCCAGGACTGAAAACTGGAA AGGTCAGAGATCAGCCAGGA	JN253442	$(TG)_6CA(TG)_4$	186	N/A	32/2	208–210	0.091	0.094	1.000
*Cac*15	TGTCCGCTAAGCTTTCGTTT ACAACCCTGATTTTGCGAAG	JN253443	$(CA)_{10}$	194	N/A	31/2	216–218	0.094	0.097	1.000
*Cac*16	TCGGAGGAACACCTCAAAAC AGTCCATCCGAAATGACAGC	JN253444	$(GT)_6$	116	N/A	32/3	136–140	0.250	0.281	1.000
*Cac*17	GCAATATTCTGCCCAAAGGA CTTGTCTGACACTGGCCTGA	JN253445	$(CT)_6$	214	N/A	32/2	232–236	0.031	0.031	1.000
*Cac*20	GCCATTGCTGCTGTTGATAA CATTTTGTTGTGTGGCCAAG	JN253447	$(GT)_6$	255	N/A	32/2	275–277	0.146	0.156	1.000
*Cac*22	TCACTGCAACTCCCACTCTG GGGAGGTAGTCGGGGAGAG	JN253448	$(TC)_{10}$	96	58	32/3	92–100	0.532	0.531	0.297
*Cac*23	AGATCCTGGCCTTCAAACCT ATGAATTCCAGCTGCTGTCC	JN253449	$(AC)_5$	161	N/A	32/2	184–186	0.031	0.031	1.000
*Cac*34	TTTGATCCTAACTTCCCTTACCA GCAGGTGGCAAGAAGCTAAC	JN253451	$(GT)_{19}$	193	54	31/11	196–216	0.804	0.871	0.632
*Cac*40	TGTCTTGCTGCAAAAAGGTG GGCGTGATCATGAATGGTTT	JN253456	$(CA)_{37}$	246	54	31/28	201–277	0.967	0.935	0.450
*Cac*42	CACACATGTACCCATGCACA AAACCTTCTTCCCTGCCACT	JN253457	$(CA)_{11}$	261	58	31/9	253–275	0.658	0.677	0.443
*Cac*45	GGGTAAATTGGAGAGGTCAGG TCACTTGTCCTGCCAGTGTC	JN253460	$(CA)_{13}$	250	58	31/7	244–260	0.531	0.581	0.441
*Cac*46	TGCACATGCTCACACATACC AAAGGGTTTGAGCTGGAAGG	JN253461	$(CA)_{11}$	222	54	31/3	216–224	0.302	0.323	0.188
*Cac*48	TTGAAGGCAAATCATTGTGG AGGTACAAGTGCTGGGATGG	JN253462	$(AC)_{13}$	162	56	32/4	151–163	0.636	0.688	0.703
*Cac*50	CATGAGCCCTGGATGTATGC TGCATTGAGACCAACCAAAG	JN253463	$(AC)_9G(CA)_6$	152	N/A	32/3	162–176	0.092	0.094	1.000
*Cac*54	AGTCTGTGACTGATCGAGGATG AGGTTTAGCTGGGACTGCTG	JN253465	$(TG)_{22}$	246	56	29/15	232–268	0.881	0.897	0.589
*Cac*56	ACCGAGATGCAAAGAGAAGG GTCTTTGGGCAAGCTGTGAG	JN253466	$(GA)_{11}$	230	56	32/5	225–231	0.658	0.656	0.645
*Cac*57	TTTATCGTCCAAAATAATGCTGAG TGGAAACATAGCGCAGTGAG	JN253467	$(TG)_{27}$	228	54	31/16	196–236	0.914	0.936	0.203
*Cac*58	TGCTTGGTACCAGTCAGCTC AGCACCCAGACACAAGTGC	JN253468	$(GT)_{18}$	184	58	32/11	186–206	0.871	0.594	0.001
*Cac*59	TTCTGGATGCTTCCAATTCC TTGTGGGAATTGCTCATGG	JN253469	$(GA)_{13}$	237	N/A	32/8	253–269	0.703	0.625	0.321
*Cac*66	CCAACACAAATTCACATGCAC CCTCCTTGAGGAAGGAAACC	JN253473	$(CA)_{14}$	219	58	32/8	208–234	0.736	0.687	0.019

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]
*Cac*67	GTAACCCATGCCTGCAGTTC CTGTCAAATTGCCGATAGGG	JN253474	$(AC)_{31}$	174	54	32/22	144–202	0.939	1.000	0.916
*Cac*68	TGCTGAGAACGGTCAGAGTG CACACACTCCACACCCAAAG	JN253475	$(TG)_7$	152	56	32/2	152–164	0.508	0.438	0.508
*Cac*B3	CCAAGACAGGAGGTGAGAGC AATCGCTCATGCAACACAAC	JN253476	$(CA)_7$	328	N/A	32/3	345–353	0.148	0.156	1.000
*Cac*B4	AAATTGCCTACTCCTGCACA AGTGCATGCGTGACTGAGAG	JN253477	$(TC)_{10}$	231	56	32/5	227–239	0.799	0.469	0.000
*Cac*B7	GGTGAGTGTGTCTTTATGAGTGC CAACGAATGCTCCAAGGTTT	JN253479	$(GT)_5$	168	N/A	29/2	194–196	0.100	0.103	1.000
*Cac*B8	GGCTGATTTTGATGTGGTGA CCCTGGAAGTTGGTATTGGA	JN253480	$(AG)_6$	241	N/A	29/3	263–281	0.508	0.379	0.161
*Cac*B11	AACTGTGGCTTTGCTTGCTT TCCCAGACTGTGGACATCAT	JN253481	$(GA)_{11}$	259	N/A	31/2	278–280	0.151	0.097	0.155
*Cac*B12	AGCTCTGCCCCGAGATAAAT AACGTGATGGGACAAATGGT	JN253482	$(CA)_9$	170	58	31/3	168–172	0.503	0.355	0.018
*Cac*B14	GCACCCTATCCTTCCCTTCT GTGCCTGTCCCAACAAGTTT	JN253484	$(AC)_{14}$	263	62	31/5	253–267	0.668	0.548	0.093
*Cac*B18	AGGGCCAGAGGAAGAAATGT AGGTTGCAGTGCTCAGGTTT	JN253485	$(GT)_5$	175	56	30/10	177–191	0.819	0.867	0.775
*Cac*B19	AGGAACTTAGGGACGCCTGT ATGCACTGTCCATACCAGCA	JN253486	$(GT)_6$	183	58	32/4	186–192	0.576	0.625	0.486
*Cac*B20	ATACACCCAAGCATGCACAC CAATCTGCCATGCCATAAAA	JN253487	$(CA)_{10}$	94	54	29/7	96–110	0.721	0.793	0.168
*Cac*B23	CAAGGTTAACCCAACGCAAT GTAGCACGCGAGAAACAATG	JN253489	$(TTGAT)_5$	282	N/A	32/3	288–298	0.304	0.344	1.000
*Cac*B28	GACATGCACATTCACAAGCA TGAGTGTGTGGCTGTGGGTA	JN253492	$(CA)_7$	110	N/A	32/2	123–127	0.198	0.219	1.000
Monomorphic microsatellites										
*Cac*7	TGCACATTGAAAATGCCCTA TGCTCCTGTGAAGCATCTTG	JN253439	$(CAA)_4$	206	N/A	29/1	244	N/A	N/A	N/A
*Cac*13	TGTCTTTCTGGGCAGCAGTA TTGCCACCACTGCAGTAAAC	JN253441	$(TG)_7$	100	N/A	32/1	121	N/A	N/A	N/A
*Cac*18	GGGATTCGAGGAATGCTACA GCTGTCAGGTAAGGCCAAAT	JN253446	$(CA)_7$	141	N/A	31/1	161	N/A	N/A	N/A
*Cac*25	CCATTTGGTGCAAGTCAACA ACTCGGCTGGACTCCCTTAC	JN253450	$(TG)_{15}$	108	N/A	30/1	150	N/A	N/A	N/A
*Cac*35	TCCTTTGAAGTGCCTTGTGA GGAGGTTTGCGCAACAAATG	JN253452	$(GTT)_5$	148	N/A	29/1	171	N/A	N/A	N/A
*Cac*36	CAGCACTGACCTGTTGTCGT CAATAACGTATCCCCGGTGT	JN253453	$(GTT)_6$	156	N/A	29/1	178	N/A	N/A	N/A
*Cac*37	TAACCGAAAGAGGTGGTGCT ATTTCATCGTGAAGGTGGTG	JN253454	$(GA)_5$	230	N/A	31/1	243	N/A	N/A	N/A
*Cac*38	GCGACATCACAGTGAAAGGA TGCACATGTACGCACTCTGA	JN253455	$(GT)_6$	269	N/A	30/1	290	N/A	N/A	N/A
*Cac*43	GGGTGCAGTGCCAGAATAAG ATGTGAGGTCTGCGTCAGTG	JN253458	$(CA)_5$	204	N/A	30/1	225	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]
*Cac*44	GGGGAGAGCTTAGGAGATGG TTCATCATGCTCTGCCAATC	JN253459	$(TG)_5$	158	N/A	32/1	180	N/A	N/A	N/A
*Cac*51	TTGTAACTATCCCAGGAGTGAGG GTTGGAGGAAATTGGAGCAC	JN253464	$(AG)_5$	234	N/A	31/1	256	N/A	N/A	N/A
*Cac*60	AGGAATGGAGCAGGGTTTTC GATGGGAGTGTGTGCATGAG	JN253470	$(CT)_5$	250	N/A	32/1	269	N/A	N/A	N/A
*Cac*63	ACGCATGAACATTCACTTGG GCTTGGCAACTGTTTCTTGG	JN253471	$(GT)_6$	236	N/A	32/1	258	N/A	N/A	N/A
*Cac*65	GACTTTCGAATGGACCTACCC TGTGTGTGTGTGCACATTGTC	JN253472	$(CA)_6$ TC $(CA)_4$	174	N/A	32/1	195	N/A	N/A	N/A
*Cac*B5	TGTTGGTTACAACGGACTGG ACTGATTGGGGAGGGAAATC	JN253478	$(TG)_5$	118	N/A	31/1	140	N/A	N/A	N/A
*Cac*B13	GGGATAGGACTGGGGACATT ACAAGAGGCCAGAAACTGGA	JN253483	$(TG)_6$	243	N/A	31/1	264	N/A	N/A	N/A
*Cac*B22	GACAGGGAGGAGAGATTGAGA TCCTGCAGTATCCCACTAGTCA	JN253488	$(GA)_6$	142	N/A	30/1	94	N/A	N/A	N/A
*Cac*B25	GGCCATAGGGCTGACTACAA CATCCAGCATTATCCAAGCA	JN253490	$(AAC)_4$	203	N/A	30/1	225	N/A	N/A	N/A
*Cac*B26	GGATGAGCGTGAGAGGTTTC TGCTTATTTGGAGGGGTTTG	JN253491	$(ACA)_4$	242	N/A	30/1	259	N/A	N/A	N/A
*Cac*B34	CTGAATTTGGAGAGGGGACA TGGCTGTTGAAGAGCAAACT	JN253493	$(CA)_7$	80	N/A	29/1	264	N/A	N/A	N/A
*Cac*B40	TGCAGTTGCTTTTGAGTTGC CCCTGGCACAGTCTTTTGTT	JN253494	$(GT)_5$	182	N/A	32/1	295	N/A	N/A	N/A
*Cac*B41	CGGGTTTTTCCTCAAAACTG GTGAGGGTCCAAAGGACAGA	JN253495	$(ATCA)_4$	154	N/A	30/1	312	N/A	N/A	N/A
*Cac*B42	ACGCTTGATAGCGCATTTTT AGCTTCACAGGCAGGAAGAG	JN253496	$(GTT)_5$	194	N/A	29/1	150	N/A	N/A	N/A
*Cac*B44	AAAGGGCAGCAATTTGTGAG ACACCTGCCAAAAGACGTG	JN253497	$(TG)_6$	173	N/A	32/1	326	N/A	N/A	N/A
*Cac*B45	TACATCATGCCTGCCACTGT AGCACCACTTGGCTTTGTCT	JN253498	$(CA)_6$	188	N/A	30/1	255	N/A	N/A	N/A
*Cac*B46	ACACGTGCACACACACTCAA GCAGGTAGCAAATGCCTGTT	JN253499	$(TTAT)_4$	320	N/A	31/1	329	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 range in base pairs of alleles

[h] H_E is expected heterozygosity

[i] H_O is observed heterozygosity

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

Integrated DNA technologies (IDT). Primer pairs yielding clean amplifications were run on 32 individuals obtained off the coast of Georgia, USA. The forward primer of 23 polymorphic microsatellites was directly labeled with either HEX or 6-FAM. To save supply costs, the 21-bp tail protocol of Boutin-Ganache et al. (2001) was used to characterize alleles at all remaining microsatellites. 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 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, 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 assess presence of null alleles, large-allele dropout, and/or stuttering at each microsatellite.

Summary data for all microsatellites (37 polymorphic, 26 monomorphic) are presented in Table 1. For polymorphic markers, the number of alleles detected ranged from two (*Cac*14, *Cac*15, *Cac*17, *Cac*20, *Cac*68, *Cac*B7, *Cac*B11, *Cac*B28) to 28 (*Cac*40); expected heterozygosity ranged from 0.031 (*Cac*17, *Cac*23) to 0.967 (*Cac*40), while observed heterozygosity ranged from 0.031 (*Cac*17, *Cac*23) to 1.000 (*Cac*67). Genotypes at *Cac*58 and *Cac*B4 deviated significantly from the expectations of Hardy–Weinberg equilibrium following sequential Bonferroni correction (Rice 1989); this was most likely due to presence of null alleles at both microsatellites, as suggested by analysis with MICROCHECKER. Single base-pair shifts were detected in the dinucleotide microsatellites *Cac*56 and *Cac*B18, but all alleles were easily scored. The microsatellites characterized in this study will prove useful for population genetic studies of *C. acronotus* and potentially for other carcharhinid species.

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