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TECHNICAL NOTE

Characterization of 11 microsatellite loci for the brown smooth-hound shark, *Mustelus henlei* (Triakidae), discovered with next-generation sequencing

Chris L. Chabot

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Abstract The brown smooth-hound shark, *Mustelus henlei* (Triakidae), is an endemic member of the eastern Pacific shark assemblage considered both commercially and recreationally important. Here, microsatellite loci for *M. henlei* discovered by next-generation sequencing (Roche 454 pyrosequencing) are described. All loci were polymorphic (3–10 alleles) with observed heterozygosities between 0.24 and 0.89 and expected heterozygosities between 0.23 and 0.86. These loci are the first to be characterized explicitly for *M. henlei* and should be useful for the investigation of population structure and gene flow in this species and for other members of the Triakidae.

Keywords Eastern Pacific Ocean · *Galeorhinus galeus* · Microsatellite · *Mustelus henlei* · Triakidae

The houndshark family, Triakidae, is a primary component of the eastern Pacific elasmobranch assemblage considered both commercially and recreationally important (Compagno 1984; Compagno et al. 2005). Several species are considered endemic to the eastern Pacific including *Mustelus henlei*, *Mustelus californicus*, *Triakis semifasciata*, *Mustelus hacat*, and *Mustelus lunulatus* (Compagno 1984; Ebert 2003; Compagno et al. 2005; Pérez Jiménez et al. 2005). Although *M. henlei* is a common component of the nearshore shark assemblage in the northeastern Pacific, there is a lack of information pertaining to the population structure and gene flow of the species. Therefore, the goal of this project was to generate a library of microsatellite markers for *M. henlei* using next-generation sequencing technology (Roche 454 pyrosequencing) in order to measure population connectivity along the eastern Pacific and provide vital information for the management and conservation of this species.

DNA used for the generation of the microsatellite library was extracted from the fin clip of an individual from Santa Barbara, California using the DNeasy blood and tissue extraction kit (Qiagen, Valencia, USA) following the manufacturer's protocols. 500 ng of DNA was prepared for whole genome shotgun sequencing on the Roche Genome Sequencer FLX instrument utilizing the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Sciences, Indianapolis, USA) following the manufacturer's protocol. The library was quantified for DNA fragment size distribution and concentration (Agilent 2100 Bioanalyzer) and then processed through the GS FLX emulsion polymerase chain reaction (PCR). Sequencing was performed in a GS FLX Titanium picotiterplate and yielded 22,309 sequences.

The sequences were screened for potential microsatellite loci by MSATCOMMANDER (Faircloth 2008) under the default settings. Of the 22,309 sequences, 1,124 contained putative microsatellite loci. Similar to the studies of the Australian gummy shark (Boomer and Stow 2010) and the tope shark (Chabot and Nigenda 2011), the majority of microsatellite motifs identified were dinucleotide in nature (68%). Primers for dinucleotide [minimum repeat number (mrn) = 8] and tetranucleotide (mrn = 4) loci were designed by PRIMER3 (Rozen and Skaletsky 2000) embedded in MSATCOMMANDER using the default settings. In total, 28 primer pairs were used for amplification trials consisting of 22 dinucleotide and six tetranucleotide loci. For all loci, the forward primer was synthesized with an M13F(−20) sequence (GTAAAACGA CGGCCAG) added to the 5′ end to incorporate a 5′ fluorescent label per the technique of (Boutin-Ganache

C. L. Chabot (✉)
UCLA, Los Angeles, CA, USA
e-mail: c.l.chabot@ucla.edu

Table 1 Characteristics of 11 microsatellite loci for *Mustelus henlei*

Locus	Forward primer 5′–3′	Reverse primer 5′–3′	Motif	N	Size (bp)	A	H_O	H_E	F_{IS}
Mh1	GGAGGAGGGAAGCCTATGG	TCTCTGGCTCCATTCAGGG	(AG)n	27	178–222	10	0.89	0.83	−0.075
Mh5	CCATTCAAAGGTCTGGTGGC	GCAGCTTCTGGCACTTGAG	(AT)n	29	302–366	10	0.55	0.86	0.361
Mh6	CATGTCCACTTCCCATCGC	GGAGAGATTAGAACAGGTGGC	(CT)n	31	191–203	6	0.44	0.58	0.242
Mh13	ACTCGATAGGCCAAAGGGC	ATGACTGGGCACCTCCAAG	(ATCT)n	32	194–212	6	0.34	0.34	−0.021
Mh15	TGCCCTTCGAGGTGGTAAG	TAGGCTGGAAAGTTGGGAG	(ATTT)n	25	416–426	4	0.24	0.23	−0.071
Mh16	GTTGATGCGGACTCACTGG	TGTCATCTGCTCCTCACCG	(GGGT)n	30	224–226	2	0.37	0.38	0.039
Mh25	TGCAATAACCGTTCTGCGTC	TCACACCCGCAGTTAGATCC	(CT)n	32	156–170	6	0.63	0.64	0.027
Mh29	ATCAGCCCAGATTGTCCGC	AGACATTCCGCCTTCCAGC	(CT)n	28	196–204	5	0.25	0.66	0.625
Mh34	CCCTTTCTAGGCTTGGCAC	CCCTCTCTCTGGAGTTGGAAG	(AG)n	30	221–225	3	0.3	0.34	0.111
Mh36	ACGATGGAGTTGACATGTATGC	ATGAGCAGCCTGGGAATGG	(AT)n	26	245–251	4	0.42	0.37	−0.155
Gg4	CTGGAATACATGCCGAGCAC	CCCGAAAGGTCTTAGTTCGC	(GA)n	27	210–214	3	0.37	0.63	0.415

N, number of samples used for characterization; Size, size of alleles; A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity

et al. 2001). Chabot and Nigenda (2011) identified nine microsatellite loci from *Galeorhinus galeus* (Gg3, Gg4, Gg7, Gg11, Gg16, Gg17, Gg18, Gg20, and Gg22) that cross-amplified in *M. henlei*. These loci were screened alongside those obtained from *M. henlei.* Initially, eight samples from Santa Barbara, California were used to test the amplification of loci and evaluate polymorphic content. The PCR protocol was as follows: A 10 μL touchdown PCR was performed using an Eppendorf Mastercycler *epgradient* S thermal cycler and the following reaction conditions: 10–100 ng template DNA, 0.2 μM reverse primer, 0.01 μM forward primer, 0.01 μM dye labeled M13 primer, 0.4 mg/mL BSA, and 5.0 μL of Qiagen Multiplex Mastermix (Qiagen, Valencia, USA). Initial denaturation was at 95°C for 15 min followed by 25 cycles of denaturation (94°C for 30 s), annealing (59°C for 90 s), extension (72°C for 60 s) and another 20 cycles of denaturation (94°C for 30 s), annealing (53°C for 90 s), extension (72°C for 60 s), and terminating with a final extension (60°C for 30 min). All PCR products were then electrophoresed on an Applied Biosystems (ABI) 3730*xl* DNA Analyzer. Allele sizes were determined by using an internal lane standard LIZ 500 (ABI) and GeneMapper® 3.7 (ABI). Out of the 37 primer pairs tested, 11 loci (10 from *M. henlei* and one from *G. galeus*) were successfully amplified by PCR and further characterized using additional samples from Santa Barbara, California (n = 24).

MICROCHECKER (Van Oosterhout et al. 2003) was used to investigate the existence of null alleles, large allele dropout, and stuttering. With the exception of Mh5, Mh29, and Gg4, all loci demonstrated a lack of null alleles. GENEPOP 4.0 (Raymond and Rousset 1995; Rousset 2008) was used to estimate allele frequencies, observed heterozygosity (HO) and expected heterozygosity (H_E), and determine departures from Hardy–Weinberg equilibrium (HWE). All 11 loci were polymorphic (3–10 alleles). H_O and H_E were 0.24–0.89 and 0.23–0.86 respectively (Table 1) and after Bonferroni correction all loci were in HWE with the exception of Mh5, Mh29, and Gg4. FSTAT 2.9.4 (Goudet 2003) was used to test for linkage disequilibrium and estimate F_{IS}. All loci were in linkage equilibrium and F_{IS} ranged between −0.155 and 0.625 (Table 1).

The development of these 10 microsatellite loci from *M.henlei* using next-generation sequencing technology, along with those of Boomer and Stow (2010) and Chabot and Nigenda (2011), should aid in the elucidation of gene flow within species of the Triakidae and provide valuable tools for the conservation of potentially threatened or exploited shark species.

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