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TECHNICAL NOTE

Characterization of 13 microsatellite loci for the tope shark, *Galeorhinus galeus,* discovered with next-generation sequencing and their utility for eastern Pacific smooth-hound sharks (*Mustelus*)

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Abstract The tope shark, *Galeorhinus galeus*, is a commercially important member of the Triakidae that has been exploited globally for the past 80 years. Here we describe 13 microsatellite loci for *G. galeus* discovered by next-generation sequencing (Roche 454 pyrosequencing) and their utility for eastern Pacific smooth-hound sharks (*Mustelus*). These loci were polymorphic (3–12 alleles) with observed heterozygosity between 0.11 and 0.86 and expected heterozygosity between 0.24 and 0.87. Several loci (7 of 13) amplified consistently for *Mustelus californicus* and *M. henlei*. These loci are the first to be characterized explicitly for *G. galeus* and should be useful in the investigation of population structure of this vulnerable elasmobranch.

Keywords *Galeorhinus galeus* · Microsatellite · *Mustelus californicus* · *Mustelus henlei* · Triakidae

The tope shark, *Galeorhinus galeus* (Triakidae), has been commercially exploited for greater than 80 years with populations demonstrating historic collapses (Ebert 2001). Continued exploitation of the species has resulted in a classification of vulnerable by the IUCN (Walker et al. 2006) and a need to determine the connectivity of globally distributed individuals in order to generate conservation strategies. Nuclear microsatellites have been used to reveal patterns of population connectivity in numerous taxa. Therefore, we have set out to generate a library of microsatellite markers for *G. galeus* using next-generation sequencing technology (Roche 454 pyrosequencing) in order to elucidate the patterns of population structure and gene flow in *G. galeus*.

DNA used for the generation of the microsatellite library was extracted from the fin clip of an Australian sample 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 with the GS FLX emulsion polymerase chain reaction (PCR) and sequencing kits. Sequencing was performed using 1/16th of a picotiterplate and yielded 40,156 sequences.

The sequences were screened for potential microsatellite loci by MSATCOMMANDER (Faircloth 2008) under the default settings. Of the 40,156 sequences, 1,344 contained putative microsatellite loci. Similar to a previous study of the Australian gummy shark (Boomer and Stow 2010), the majority of microsatellite motifs identified were dinucleotide in nature (~80%). Primers for dinucleotide (minimum repeat number (mrn) = 8), tetranucleotide (mrn = 4), and pentanucleotide (mrn = 4) loci were designed by the PRIMER3 software (Rozen and Skaletsky 2000) embedded in MSATCOMMANDER using the default settings. In total, 32 primer pairs were used for amplification trials consisting of 18 dinucleotide, 11 tetranucleotide, and 2 pentanucleotide 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 et al.

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2001). Initially, eight samples from four subpopulations (2 samples per population: North America (California), South Africa, Australia (Australian Bight and Tasmania), and the U.K. (Irish Sea)) were used to test 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 32 primer pairs tested, 13 were successfully amplified by PCR and further characterized using additional samples from the Australian Bight and Tasmania ($n = 28$). In order to validate the dataset, 30% of our samples were reanalyzed at all loci producing identical genotypes between reads.

MICROCHECKER (Van Oosterhout et al. 2003) was used to investigate the existence of null alleles, large allele dropout, and stuttering. With the exception of Gg2, Gg17, Gg18, and Gg22, 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 (H_O) and expected heterozygosity (H_E), and determine departures from Hardy–Weinberg equilibrium (HWE). All 13 loci of *G. galeus* were polymorphic (3–12 alleles). H_O and H_E were 0.11–0.86 and 0.24–0.87 respectively (Table 1) and after Bonferroni correction all loci were in HWE with the exception of Gg4 and Gg17. 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 −0.132 and 0.721 (Table 1).

To determine the utility of these markers for genotyping species of eastern Pacific smooth-hound sharks (*Mustelus*), we tested the 13 loci on *Mustelus californicus* and *M. henlei* using the PCR reactions and analyses described above. Seven of the loci successfully amplified for both species and two loci produced stutter products (Table 2). The development of these 13 microsatellite loci from *G. galeus* using next-generation sequencing technology, along with those of Boomer and Stow (2010), should aid in the elucidation of gene flow within species of the Triakidae

Table 1 Characteristics of 13 microsatellite loci for *Galeorhinus galeus*

Locus	Forward primer 5′–3′	Reverse primer 5′–3′	Motif	N	Size (bp)	A	H_O	H_E	F_{IS}
Gg2	TGGCTCAGTCCAGAAACCC	CCCTATTCGAGAGGCCCAG	$(TG)_n$	29	249–259	6	0.30	0.55	0.336
Gg3	CCGTGACTGAAAGCAGCC	CCCTCAACCATGGCAAGTG	$(GATT)_n$	28	257–265	4	0.43	0.46	0.128
Gg4	CTGGAATACATGCCGAGCAC	CCCGAAAGGTCTTAGTTCGC	$(GA)_n$	29	179–213	3	0	0	NA
Gg7	CTGTGGAACCAAACTCCAGC	AGCTGGTCGAGGTGAATGC	$(AG)_n$	29	296–312	5	0.48	0.51	0.060
Gg11	AAGTTGCACGTTTCCCAGC	TACTGCAGGACCGGTTTCC	$(TCCC)_n$	28	329–363	8	0.68	0.60	−0.132
Gg12	TGTCAAACACCATCGCAGG	TGCTCTGAAGTCTACAAGAATGG	$(TA)_n$	25	276–296	11	0.70	0.72	0.024
Gg15	GGCTGAATGGTTTCCCAGC	GCCTCCAACTTAGCATAGCC	$(GA)_n$	27	147–169	12	0.85	0.87	0.027
Gg16	AGTGTGGTCTCACCAATGC	TGGAAGGGTAAGGAAATTGGC	$(GA)_n$	27	174–182	3	0.41	0.43	0.047
Gg17	CCTGCTTGTGACAGTTACCC	ACAGGCATCACCTCTGTGC	$(AC)_n$	27	159–181	5	0.15	0.52	0.721
Gg18	TCCACTTCAGGAAGGCCAG	CAAAGCCAGGTGGTTCTCC	$(GA)_n$	28	179–187	4	0.11	0.31	0.661
Gg20	GACCAAGGGTCATCCAGAC	TCAGCTTGGGCAATTCCAG	$(TC)_n$	29	194–202	3	0.21	0.24	0.147
Gg22	TCCTGGGATGGCAACTTCG	AGGCCACCCAACTATCCTG	$(GT)_n$	30	237–247	7	0.63	0.82	0.229
Gg23	ACAGACCACAGGGCATGG	TGCAGAGCAGGCTAGATGG	$(AC)_n$	28	258–278	9	0.86	0.83	−0.029

N number of Australian samples, *Size* based on all samples, *A* number of alleles, H_O observed heterozygosity for Australian samples, H_E expected heterozygosity for Australian samples

Table 2 PCR results of the 13 loci for *Mustelus californicus* and *M. henlei*

	Gg2	Gg3	Gg4	Gg7	Gg11	Gg12	Gg15	Gg16	Gg17	Gg18	Gg20	Gg22	Gg23
Mustelus californicus	S	0	+	+	+	0	0	+	+	+	+	S	0
Mustelus henlei	S	+	+	+	+	0	0	+	+	+	+	+	0

Successful PCRs indicated by +, stutter products by S, and failed reactions by 0

and provide valuable tools for the conservation of threatened and data deficient shark species.

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