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MICROSATELLITE LETTERS

Characterisation of 15 novel microsatellite loci for the grey reef shark (*Carcharhinus amblyrhynchos*)

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Abstract Grey reef sharks (*Carcharhinus amblyrhynchos*) are important apex predators on coral reefs, and their numbers have declined dramatically as a result of overfishing. Knowledge of environmental factors that shape gene flow is essential for developing appropriate management strategies, but the lack of suitable genetic markers has hindered research on this species. Here, we characterised 15 polymorphic microsatellite loci for grey reef sharks. None of the loci deviated significantly from Hardy–Weinberg equilibrium and there was no evidence of Linkage Disequilibrium. Several loci cross-amplified in other carcharhinid species, and will be useful in future studies of this family.

Keywords Grey reef sharks · *Carcharhinus amblyrhynchos* · Microsatellites · Coral reef

Grey reef sharks (*Carcharhinus amblyrhynchos*) exert important top-down control on coral reef ecosystems and their removal may accelerate reef degradation through trophic cascades. Grey reef sharks are categorised globally as a "Near Threatened" species (Smale 2009) however dramatic regional declines, even in areas such as the comparatively well managed Great Barrier Reef, suggest that some populations may be under substantially higher levels of risk (Robbins et al. 2006). Understanding what environmental factors shape gene flow is essential for the development of effective management strategies, but the lack of appropriate genetic markers has thus far hindered research effort in that direction. Here we report the isolation of 15 novel, polymorphic microsatellite markers for the grey reef shark.

Genomic DNA was extracted from a fin clip of a male grey reef shark using the ISOLATE II Genomic DNA Kit (Bioline Pty Ltd) following manufacturer instructions. Pyrosequencing was performed on a 454 GS-FLX platform (Roche Applied Science), resulting in 186,119 sequences and 68,169,247 bases. A total of 1,882 microsatellite loci (di -tri - tetra -penta- and hexa-nucleotides with more than 5 repeats) with flanking regions suitable for primer design were identified with QDD v 2.0 (Meglécz et al. 2010). Of these loci, 28

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Table 1 Novel microsatellite loci isolated in this study

Locus	Primer sequence (forward and reverse)	Motif	Size (bp)	N	NA	H_O (H_E)	H–W	PID
C.amb2	F-TCCTACCTGACAAAGGAACTGC[4]	$(TCTA)_{14}$	328–368	31	11	0.90 (0.87)	0.761	0.029
	R-ATGAACAGAGACAAACAGACCGAC							
C.amb3	F-TGGAGTGCCAATTCTCTTGTCG[1]	$(TC)_{18}$	200–272	34	26	0.82 (0.90)	0.031	0.013
	R-ACTTGGGAGTCTGACTAATCTCC							
C.amb4	F-GTCGAATGCATTGAGTTTCAGG[2]	$(AC)_{14}$	346–372	32	13	0.78 (0.83)	0.335	0.049
	R-CCAATACAAGCAAAGGGACAAC							
C.amb5	F-CAGATATGCGGTGTCGTGGC[1]	$(TG)_{13}$	266–284	33	8	0.70 (0.71)	0.202	0.117
	R-TTCCGCGTCTTGTCTCTGC							
C.amb6*	F-TGTGGCTGGGATAAAATGCACG[3]	$(TGG)_{13}$	251–287	32	11	0.90 (0.81)	0.580	0.054
	R-TGGCTTGTAAAATCCTGTTCTGCG							
C.amb7	F-AGAATGCTGTCTCGTGATGC[3]	$(AGAC)_{11}$	291–315	34	7	0.79 (0.75)	0.039	0.095
	R-GTTGTCACTGTCGAGATAGAGC							
C.amb9	F-CCCAGGAGCCCTCTCTGTA[4]	$(TG)_{13}$	209–223	34	6	0.56 (0.58)	0.885	0.254
	R-GTCTCTTGCCACGCTCCTAC							
C.amb11	F-TGAACGCTTTACTGAACCTTGC[3]	$(CA)_{14}$	162–200	33	14	0.90 (0.88)	0.373	0.024
	R-GCAGCCTTTACTCCTCGTCA							
C.amb15	F-GTATGAGACGAGCATCGTGCC[2]	$(AC)_{13}$	192–222	33	12	0.88 (0.86)	0.38	0.034
	R-AATCGCAGCGTCTGCAATG							
C.amb18	F-TGCACACGCAGTGATGTTGG[2]	$(AC)_{16}$	143–191	31	21	0.97 (0.93)	0.058	0.008
	R-ATGCCGATTTCTCTGTTAATGAGC							
C.amb20	F-ATGTGGAGGAGTGATGTTAGCC[1]	$(GT)_{12}$	314–350	31	14	0.87 (0.89)	0.705	0.021
	R-TTAATGTCAGTGTTCACGCTGG							
C.amb22	F-ATGTCAGTTCTTTAGGAGTAGGG[1]	$(GA)_{11}$	352–356	32	3	0.22 (0.20)	1	0.652
	R-CCAATCTACACTTCACTCACTG							
C.amb25*	F-GACTCATCAGGATAGTCTGGATGCT[1]	$(AGGG)_8$	208–248	32	10	0.75 (0.79)	0.214	0.071
	R-GCTCAACTGTCAAAAGAGGAAGCC							
C.amb27*	F-AGTCAGTGTCACGATGG[4]	$(TG)_{11}(AG)_{18}$	169–197	33	10	0.82 (0.83)	0.483	0.047
	R-GCTTTCTATCATTAACATGAGATCC							
C.amb28*	F-CACATTGCTATGAGCCTGGAG[2]	$(AC)_{13}$	286–326	31	10	0.77 (0.75)	0.767	0.082
	R-CATCTCTTTCATCACTGCATGATTG							

N = sample size, NA = number of alleles, H_o = observed heterozygosity, H_E = expected heterozygosity, H–W = p value for Hardy–Weinberg Equilibrium (none significant after Holm–Bonferroni sequential correction), PID = non exclusion Probability of Identity. The fluorescent dye used is indicated in superscripts: [1] 6-FAM, [2] VIC®, [3] NED®, [4] PET®. Loci amplified using the M13 tail protocol (Schuelke 2000) are indicated with *. Combined PID = 7.31×10^{-20}

were chosen following the recommendations from Gardner et al. (2011). Primers were designed with similar annealing temperatures (55–60 °C) and for primer compatibility to increase multiplexing potential. All forward primers were labelled with fluorescent dyes (6-FAM, PET®, NED® and VIC®) with the exception of C.amb6, C.amb25, C.amb27 and C.amb28 which were genotyped using the M13-tail approach (Schuelke 2000). Primers were tested on 34 individuals from the northern Great Barrier Reef (Lizard Island, QLD Australia). Microsatellite loci were amplified using the Type-it Microsatellite PCR Kit (Qiagen) following manufacturer instructions. PCR conditions were as follows: 5 min denaturation at 95 °C, followed by 35 cycles of 30 s denaturation (94 °C), 30 s annealing (60 °C) and 30 s elongation (72 °C) and a final elongation of 10 min at 72 °C. For M13 tailed primers, annealing temperature followed a touch-down protocol, starting at 60 °C and decreasing by 0.5 °C per cycle for the first 20 cycles.

Fragment analysis was performed on an ABI 3730 platform at the Sydney node of the Australian Genome Research Facility. Fifteen loci amplified consistently and showed no sign of null alleles, large allele drop-out or excessive stuttering. Estimates of genetic diversity were obtained and tested for Hardy–Weinberg Equilibrium (H–W) and linkage disequilibrium (LD) using the software package Genepop 4.2 (Rousset 2008) and Probability of Identity (PID) for each

locus was estimated in Cervus 3.0.3. After applying Holm-Bonferroni sequential correction for multiple comparisons all loci were in linkage equilibrium and none departed significantly from H–W (Table 1). The average number of alleles per locus was 12.6 (range 3–26), mean observed (H_O) and expected (H_E) heterozygosities were 0.785 (range 0.22–0.97) and 0.783 (range 0.2–0.95) respectively (Table 1). The 15 novel loci successfully cross-amplified for a range of other carcharhinid species (Table S1). The loci developed in this study will prove useful for future investigations on the genetic structure of grey reef sharks as well as other Carcharhinidae.

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