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MICROSATELLITE LETTERS

Isolation and characterization of fifteen microsatellite loci in Leopard grouper (*Mycteroperca rosacea*) via 454 pyrosequencing

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Abstract Leopard grouper (*Mycteroperca rosacea*) are distributed from Bahía Magdalena, through the Gulf of California and as far south as Jalisco, Mexico. They are listed as Vulnerable on the IUCN Red List due to significant fishing-induced declines over the past decade. Microsatellite loci were isolated by constructing a shotgun genomic library and sequencing using 454 XL + titanium chemistry. We characterized 15 polymorphic loci in 120 samples from three geographic locations. Genotyping yielded 15–60 alleles per locus with observed levels of heterozygosity ranging from 0.575 to 0.975.

Keywords Leopard grouper · *Mycteroperca rosacea* · Microsatellites · Fisheries management · Conservation

Primer note text

Leopard grouper (*Mycteroperca rosacea)* are distributed from Bahía Magdalena, through the Gulf of California and as far south as Jalisco, Mexico (Thomson et al. 2000). They are listed as Vulnerable on the IUCN Red List due to significant population declines resulting from overexploitation of individuals from spawning aggregations (Craig and Sadovy 2008). We isolated and characterized fifteen polymorphic microsatellite loci to facilitate a better understanding of the genetic diversity and population structure of a commercially exploited species. Additionally, polymorphic loci will be used to validate patterns of larval connectivity among sites derived from oceanographic models.

We constructed a shotgun genomic library using ~5 μg of genomic DNA from a single individual, which was sequenced using 454 XL + titanium chemistry (Roche Applied Science, Indianapolis, IN, USA) at the University of Arizona Genetic Core. We generated over 142,733 unique reads with an average length of 595.46 bp (range = 34–1,091, mode = 770 bp) after quality filtering (Q ≥20 using a 10 bp sliding window). We located di-nucleotide microsatellite loci containing at least 15 perfect repeats and tetra-nucleotide loci with at least 8 perfect repeats and designed primers using the software QDD (Meglécz et al. 2010). We used unique sequence reads larger than 130 bp and consensus sequences within contigs grouping sequences with a similarity ≥95 % in

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Table 1 Primer sequences and characteristics of 15 microsatellite loci for leopard grouper (*Mycteroperca rosacea*)

Locus accession no.	Motif	Primer (5′–3′)	No. of alleles	Size range (bp)	Bahía de los Angeles $H_o(H_e)$	Guaymas $H_o(H_e)$	La Paz $H_o(H_e)$
Mros01[a]	$(GT)_{44}$	F: CACTGTCACAAATGGCATCC	35	154–234	0.775 (0.970)[b]	0.775 (0.956)[b]	0.750 (0.964)[b]
		R: AGTCCACTGTCCACTGTCCC					
Mros02[a]	$(GT)_{44}$	F: TCACCACTCGGGAGTTGTT	51	228–348	0.825 (0.967)[b]	0.900 (0.937)	0.875 (0.953)[b]
		R: TCACTTTAGGGACGTGAGGG					
Mros03	$(GT)_{43}$	F: CCATCATGAAGCTTTGACCA	25	104–160	0.885 (0.911)	0.950 (0.915)	0.800 (0.919)
		R: TTGACTTTATCTCCAAGGCAAA					
Mros04	$(GT)_{39}$	F: TGGTGTGATGATTGTTGCTG	31	95–157	0.925 (0.956)	0.935 (0.960)	0.925 (0.943)
		R: GATGACAGACATGATGGCCT					
Mros05	$(CA)_{36}$	F: GGGACCTGAATGAGATCAACA	33	117–199	0.850 (0.947)	0.925 (0.959)	0.950 (0.944)
		R: ATCCTCAAGGACTGCTGGTG					
Mros06[a]	$(CA)_{35}$	F: TTTCACCATGTTAGTAATTCAGTTCA	29	82–152	0.625 (0.924)[b]	0.750 (0.951)[b]	0.625 (0.949)[b]
		R: TAAGGCCAATCACTGCTGCT					
Mros07	$(GT)_{36}$	F: CATTAGTGCTGCAAGGCTCA	35	140–216	0.925 (0.948)	0.950 (0.950)	0.950 (0.962)
		R: CAGTGAAAGGCTTGGTGTCA					
Mros08[a]	$(GT)_{36}$	F: TCATCCTTCACCATCAACCA	31	138–206	0.675 (0.941)[b]	0.600 (0.938)[b]	0.575 (0.940)[b]
		R: GCTGAGATTTCAGCATTCA					
Mros09	$(GT)_{36}$	F: GCACGAGCAAGTCTTTGTGA	31	145–211	0.950 (0.945)	0.925 (0.956)	0.900 (0.944)
		R: CGGTCGACCTTTGTAGCTGT					
Mros10	$(TAGA)_{33}$	F: ACAAATGCGAGTGAAACGCT	42	161–353	0.975 (0.961)	0.925 (0.970)	0.925 (0.960)
		R: CACCTCTGTCAACTTGGCAAT					
Mros11	$(TCTA)_{19}$	F: ATCGAGACGAAAGGATGCAG	20	83–181	0.875 (0.914)	0.925 (0.922)	0.950 (0.926)
		R: TCCGTCAGCAGTTTACTCCC					
Mros12	$(TAGA)_{16}$	F: GTCCTGCACTCAGCTTCCTC	24	186–302	0.875 (0.937)	0.900 (0.939)	0.950 (0.933)
		R: TTCCATGACTGATCCAGCCT					
Mros13	$(GT)_{43}$	F: ACAGTTTCCTCCACCAGCAT	60	125–275	0.975 (0.977)	0.950 (0.981)	0.960 (0.979)
		R: GTGGGGCCAAGCTACTGTAA					
Mros14	$(GATT)_{13}$	F: ATAGCCCCTCTGAGGCAAAT	15	146–226	0.852 (0.863)	0.851 (0.883)	0.765 (0.858)
		R: TTGCAAATCCAAATAAGTGTTT					
Mros15	$(TCTA)_{13}$	F: GGAGACGGTTCAGAGAACA	21	151–235	0.923 (0.925)	0.925 (0.911)	0.897 (0.914)

[a] Denote loci with null alleles. [b] Denote loci that deviate from Hardy–Weinberg equilibrium

regions ≥30 bp flanking repeats to design primers. The aforementioned step eliminated duplicated loci that have diverged in the flanking regions (except recent duplicates) and reduced null alleles by assembling sequence data from those regions with coverage ≥1 × for anchoring primers. We obtained 431 di-nucleotide and 65 tetra-nucleotide microsatellite loci that met our criteria.

We collected 120 tissue samples from sites distributed across the geographic range of Leopard grouper, including Bahía de Los Angeles, Guaymas and La Paz (40 individuals per locality). DNA was extracted using standard chloroform extraction protocols (Sambrook et al. 1989). PCR reactions were set up following protocols associated with the Multiplex PCR kit (Qiagen). We tested primer pairs for 20 di-nucleotide and 10 tetra-nucleotide microsatellite loci. Successfully amplified loci were labeled with 6FAM, HEX, NED and PET and combined into four groupings: Multiplex A (*Mros*01, *Mros*06, *Mros*07, *Mros*10, *Mros*11, *Mros*12), Multiplex B (*Mros*02, *Mros*03, *Mros*05, *Mros*08, *Mros*09), Multiplex C (*Mros*13, *Mros*14, *Mros*15) and locus *Mros*04. PCR reaction volume was 10 µl, with a final concentration of 1 × for the Qiagen Multiplex master mix and 0.2 µM for primers. PCR amplification of microsatellite loci was performed on a GeneAmp PCR System 9,700 (Applied Biosystems) using the following parameters: an initial hold at 94 °C/15 min, 35 cycles of 94 °C/30 s, 60 °C/90 s, 72 °C/60 s, with a final extension of 72 °C/30 min. PCR products were sized on an ABI 3730xl DNA sequencer (Applied Biosystems). Microsatellites were analyzed using standard analyses protocols described in Jackson et al. (2012).

Fifteen primer pairs amplified polymorphic products. Genotyping 120 samples for all 15 microsatellite loci

yielded 15–60 alleles per locus with observed levels of heterozygosity ranging from 0.575 to 0.975 (Table 1). There was evidence of null alleles for four loci (*Mros*1, *Mros*2, *Mros*6, *Mros*8) based on an excess of homozygotes. A cut-off allele frequency is derived in Micro-Checker based on the equation, $p > (2Nq\text{-null})^{-1}$, where p is the allele frequency, N is the number of samples analyzed and q-null is the estimated null allele frequency. No significant linkage disequilibrium was observed between markers in all three populations ($p > 0.05$, after Bonferroni correction). Exact tests revealed that only 11 of 45 estimates of expected heterozygosity deviated from Hardy–Weinberg equilibrium (HWE) ($p < 0.05$). Loci deviating from HWE largely coincided with the presence of null alleles. While deviations from HWE are likely due to the presence of null alleles, deviations from HWE may also be explained by selection, incomplete sampling, mating system effects or fluctuations in population size. Microsatellites developed here will assist with population genetics studies and implementation of spatially explicit fisheries management strategies for *M. rosacea* in the Gulf of California, Mexico.

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