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TECHNICAL NOTE

Isolation of microsatellite markers from tiger grouper (*Mycteroperca tigris*) and characterization in yellowfin grouper (*Mycteroperca venenosa*), coney (*Cephalopholis fulva*), and red hind (*Epinephelus guttatus*)

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Abstract Thirty-six microsatellite markers were isolated from an enriched genomic library of tiger grouper (*Mycteroperca tigris*) and characterized in 20 individuals each for yellowfin grouper (*Mycteroperca venenosa*), coney (*Cephalopholis fulva*), and red hind (*Epinephelus guttatus*). The microsatellites include 23 dinucleotide repeats, twelve trinucleotide repeats, and one tetranucleotide repeat. The microsatellites characterized in this study will be useful for further evaluation of population-genetic indices in the economically important subfamily Epinephelinae.

Keywords Microsatellites · *Mycteroperca tigris* · *Mycteroperca venenosa* · *Cephalopholis fulva* · *Epinephelus guttatus*

Introduction

The tiger grouper, *Mycteroperca tigris*, a western Atlantic member of the economically important subfamily Epinephelinae (groupers), is distributed from southern Florida and Bermuda to southern Brazil (Heemstra and Randall 1993). Tiger grouper are susceptible to increased fishing pressure targeted at spawning aggregations, a shared concern among several grouper species (Sadovy et al. 1994; Sadovy de Mitcheson et al. 2008). The population density of tiger grouper is generally sufficiently low such that they are rarely caught except when spawning in large aggregations (Robinson et al. 2011). Large bodied groupers, such as tiger groupers, recently have been proposed as potential biocontrol agents for invasive lionfish in the Caribbean Sea (Maljkovic et al. 2008; Mumby et al. 2011). Given the potential for tiger grouper (and other large bodied groupers) to mitigate the alarming spread of lionfish in Caribbean waters, the conservation of these species is of more than passing interest.

Nuclear-encoded microsatellite markers are ideal tools for assessments of population-genetic indices, including genetic variability and the identification of stock-structure (Ward 2000).These markers allow for the evaluation of indices both within and between spawning aggregations, identifying locations that are critical for conservation efforts. Microsatellites can also enable the measurement of larval dispersal (Planes et al. 2009) and estimate the optimal sizing and spacing for conservation zones such as marine protected areas (MPAs). Herein, we describe polymerase chain reaction (PCR) primers for 36 microsatellite markers isolated from an enriched tiger grouper genomic library.

Specifics pertaining to the production of the enriched microsatellite library can be found in Renshaw et al. (2011). Whole genomic DNA was extracted from a single tiger grouper (*M. tigris*) using the DNeasy® Blood and Tissue Kit (Qiagen). Independent dinucleotide and trinucleotide microsatellite enrichments were ligated into pCR® 2.1-TOPO® vectors (Invitrogen), and transformed into *Escherichia coli* (One Shot® TOP10 Chemically Competent Cells, Invitrogen). Positive (white) colonies were handpicked with sterile toothpicks, placed in 96-well tissue culture plates with 200 µl LB broth (containing 50 µg/ml

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Table 1 Summary data for 36 microsatellite markers isolated from tiger grouper (*M. tigris*) and characterized in yellowfin grouper (*M. venenosa*), coney (*C. fulva*), and red hind (*E. guttatus*)

Microsatellite	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone Size[d]	Species[e]	N/N_A^f	Size Range[g]	H_E/H_O^h	P_{HW}^i
*Mti*4	TGGGATGAAGTTGGAGAAGG[NED]	JX091787	$(GAT)_6$	232	*Mve*	20/1	252	0.000/0.000	1.000
	CCCTGAAAATGGAGTTGTGC				*Cfu*	20/8	267–294	0.837/0.800	0.512
					Egu	20/4	255–273	0.449/0.350	0.308
*Mti*7	TGCTGTAAAGCCCTGTGAGG[FAM]	JX091788	$(ATTG)_{10}$	333	*Mve*	20/5	347–371	0.641/0.550	0.410
	CAATCACTGCTTCTGAACTGC				*Cfu*	20/1	279	0.000/0.000	1.000
					Egu	X	X	X	X
*Mti*9	AGAGCTGGATACAGCGTTGG[FAM]	JX091789	$(CAT)_{14}$	323	*Mve*	20/3	329–341	0.497/0.600	0.503
	ATTGGTGAATGGCACTAGCC				*Cfu*	X	X	X	X
					Egu	X	X	X	X
*Mti*10	TGAGTCAGTCTTCTTTGGATGC[HEX]	JX091790	$(TCA)_{11}$	165	*Mve*	X	X	X	X
	TCAGATAGTAATTTTGTCATGTATTGC				*Cfu*	X	X	X	X
					Egu	20/8	167–188	0.833/0.500	**0.002**
*Mti*11	TCAGATAGTAATTTTGTCATGTATTGC[FAM]	JX091791	$(GAT)_{16}$	290	*Mve*	18/5	287–317	0.768/0.722	0.763
	GGCTGACATTGTGGTTTTGG				*Cfu*	X	X	X	X
					Egu	X	X	X	X
*Mti*12	GCTGCAGAGGGACAGATACC[NED]	JX091792	$(AC)_{50}$	259	*Mve*	20/8	225–273	0.841/0.900	0.223
	CACAGCGTATCCCTGTTCG				*Cfu*	X	X	X	X
					Egu	20/18	231–307	0.937/0.750	**0.002**
*Mti*14	CGGTGCAGTGTGTTCAGC[FAM]	JX091793	$(TGA)_6$	340	*Mve*	18/3	362–371	0.452/0.500	1.000
	TAGTGTCCTGCATGATGAGC				*Cfu*	20/1	361	0.000/0.000	1.000
					Egu	X	X	X	X
*Mti*16	TTTGGAAAGAATCCATGTGC[NED]	JX091794	$(CAT)_6$	223	*Mve*	20/3	225–246	0.145/0.150	1.000
	ATCTGGCAGACGGTTAATGG				*Cfu*	20/2	228–255	0.050/0.050	1.000
					Egu	20/2	227–230	0.262/0.300	1.000
*Mti*17	TCACTGAGGGCTACTGTGATG[HEX]	JX091795	$(TGA)_8$	187	*Mve*	20/5	190–205	0.451/0.500	0.253
	ACAGCTGGGAAACAAACCAG				*Cfu*	20/1	141	0.000/0.000	1.000
					Egu	X	X	X	X
*Mti*18	AACACGCTGAGTCTGTACGC[HEX]	JX091796	$(TGA)_{14}$	177	*Mve*	20/3	175–181	0.188/0.200	1.000
	CCTGTCTTCGTCTTCACATCG				*Cfu*	X	X	X	X
					Egu	X	X	X	X
*Mti*21	TCCTGATCTGAGCGACTACG[FAM]	JX091797	$(CAT)_{14}$	332	*Mve*	20/6	353–368	0.633/0.550	0.327
	GAAATGAGGCAGATTTCAGG				*Cfu*	X	X	X	X
					Egu	20/7	334–352	0.769/0.750	0.117
*Mti*22	CTTAGTGACCAGCCACTGTCC[HEX]	JX091798	$(TGA)_7$	182	*Mve*	20/2	200–203	0.328/0.300	1.000
	CTTAGTGACCAGCCACTGTCC				*Cfu*	20/6	205–220	0.712/0.750	0.937
					Egu	20/2	202–205	0.224/0.250	1.000
*Mti*23	TGATGGATCTCATGGAGTCG[FAM]	JX091799	$(ATG)_5$	296	*Mve*	20/2	311–317	0.185/0.200	1.000

Table 1 continued

Microsatellite	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone Size[d]	Species[e]	N/N_A^f	Size Range[g]	H_E/H_O^h	P_{HW}^i
	CGGCAACATTGTGTTAGTGC				*Cfu*	X	X	X	X
					Egu	X	X	X	X
*Mti*24	ATTGGAAAGCAGGAACTTGG[NED]	JX091800	$(GT)_{25}$	205	*Mve*	20/19	209–281	0.935/0.900	0.214
	CAGACGCGCACATATACATAGC				*Cfu*	20/23	235–295	0.956/0.950	0.398
					Egu	X	X	X	X
*Mti*25	AGAGGAGTGCAGTGTTGAGG[FAM]	JX091801	$(ATG)_6$	329	*Mve*	X	X	X	X
	ATCAGCTGTGACCACTGACG				*Cfu*	X	X	X	X
					Egu	20/6	296–362	0.321/0.250	0.102
*Mti*31	CTCTCACATATTCACACAGACAGC[NED]	JX091802	$(AC)_9$	216	*Mve*	20/12	238–288	0.869/0.850	0.330
	ACTGCGGCATTCATTAGACC				*Cfu*	20/2	231–233	0.050/0.050	1.000
					Egu	20/22	244–306	0.953/0.850	0.052
*Mti*33	CGGGATTTTAACTCGCTTCC[FAM]	JX091803	$(TG)_{14}$	257	*Mve*	20/7	271–285	0.786/0.700	0.838
	AGCAGCAAGAATGTCACAGG				*Cfu*	X	X	X	X
					Egu	X	X	X	X
*Mti*34	GCATTGCTGTGATCTAGTGTCC[FAM]	JX091804	$(CA)_{32}$	232	*Mve*	20/10	222–242	0.847/0.800	0.405
	GGGTCTGAGCCTCTGTAAGC				*Cfu*	20/8	209–223	0.744/0.750	0.554
					Egu	20/20	220–298	0.962/1.000	1.000
*Mti*35	GCCAGGAGTAACCAATCACC[HEX]	JX091805	$(GT)_{11}$	133	*Mve*	20/4	145–153	0.191/0.200	1.000
	TTTTCTGTTTCCACATAAAACTGG				*Cfu*	20/25	162–224	0.977/0.950	0.096
					Egu	18/8	155–205	0.659/0.611	0.443
*Mti*37	GTTTCACGCCTCCTCTATCG[NED]	JX091806	$(CA)_{10}$	175	*Mve*	20/1	191	0.000/0.000	1.000
	CTGCTCGAGTTTCATCATGG				*Cfu*	20/11	182–222	0.890/0.900	0.813
					Egu	X	X	X	X
*Mti*39	AACCCCTGCTGAGTAAATGC[HEX]	JX091807	$(TG)_{35}$	144	*Mve*	20/20	132–174	0.962/0.900	0.055
	AGTGGGCGAACAAACACC				*Cfu*	20/15	118–176	0.936/0.950	0.797
					Egu	20/21	119–181	0.958/1.000	0.146
*Mti*41	GGATAAACTGATAAGGTTGTCTGAGG[HEX]	JX091808	$(TG)_{19}$	89	*Mve*	20/4	128–136	0.529/0.550	0.304
	AAACAGATATCTTGGTTCATTGC				*Cfu*	20/12	97–141	0.873/0.800	0.660
					Egu	X	X	X	X
*Mti*42	ATTCACATCCTCCCATGACC[FAM]	JX091809	$(AC)_{22}$	257	*Mve*	20/12	252–290	0.844/0.850	0.749
	CGCAGTTTGTTACATGCTAAGG				*Cfu*	X	X	X	X
					Egu	19/3	247–255	0.104/0.053	0.028
*Mti*43	TTGCGGGTTAATATCAGAGG[NED]	JX091810	$(TG)_{15}$	157	*Mve*	20/8	168–184	0.773/0.650	0.187
	AACGCCTCTACCATGACAGC				*Cfu*	20/5	161–177	0.546/0.500	0.054
					Egu	20/12	166–188	0.865/0.700	0.207
*Mti*44	GCGGGTATCAACACAATGG[FAM]	JX091811	$(TG)_{13}$	195	*Mve*	20/5	217–225	0.606/0.450	0.060
	CCTACCTCCCTGCATAATGG				*Cfu*	20/7	219–245	0.676/0.600	0.426

Table 1 continued

Microsatellite	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone Size[d]	Species[e]	N/N_A^f	Size Range[g]	H_E/H_O^h	P_{HW}^i
					Egu	20/13	232–276	0.912/0.900	0.859
*Mti*45	CTCCTGGTTTTCCCATTGC^{HEX}	JX091812	$(TG)_{32}$	194	*Mve*	20/3	168–172	0.188/0.200	1.000
	AGAGGGACATCCTGTCATGG				*Cfu*	18/3	176–180	0.110/0.111	1.000
					Egu	20/5	175–187	0.677/0.600	0.243
*Mti*49	CACACAGGAAAGTGAGAATTGC^{NED}	JX091813	$(AC)_{24}$	186	*Mve*	20/8	178–214	0.773/0.750	0.269
	TCTAACAGAAAACAAAACATCTGG				*Cfu*	X	X	X	X
					Egu	20/14	185–215	0.888/0.900	0.142
*Mti*50	CAGCGATTACATGCCTTACG^{HEX}	JX091814	$(TG)_{15}$	103	*Mve*	20/8	117–145	0.762/0.800	0.681
	GGGTCACTTGTCATTTGTGC				*Cfu*	X	X	X	X
					Egu	20/4	118–124	0.545/0.650	0.722
*Mti*51	CCAGCATATGGAAAACAATAAATC^{FAM}	JX091815	$(TG)_{16}$	193	*Mve*	20/17	202–270	0.941/1.000	1.000
	CCAAGTTAGTGCCAAACTGC				*Cfu*	X	X	X	X
					Egu	20/1	243	0.000/0.000	1.000
*Mti*52	GCTGCTGTTTCCTTTCTCTCC^{HEX}	JX091816	$(GT)_{35}$	192	*Mve*	20/4	166–176	0.556/0.500	0.716
	GCTTTGAAGCAGAACCATGC				*Cfu*	X	X	X	X
					Egu	20/15	183–223	0.917/0.950	0.980
*Mti*53	TGGACTTCCAAGACAGTGAGC^{HEX}	JX091817	$(TG)_{28}$	173	*Mve*	20/10	170–194	0.854/0.900	0.293
	ACGATACCTCTGGGTCAAGC				*Cfu*	20/1	144	0.000/0.000	1.000
					Egu	20/12	173–205	0.923/0.800	0.223
*Mti*55	TGAACATCAGGAAAAACACAAAAC^{FAM}	JX091818	$(GT)_{32}$	200	*Mve*	20/8	185–199	0.805/0.850	0.948
	TGGAACTCTCGTCTGTCTCATC				*Cfu*	X	X	X	X
					Egu	X	X	X	X
*Mti*56	TTCTTAGTTTTTCATCCGACAGC^{NED}	JX091819	$(CA)_{11}$	199	*Mve*	20/1	215	0.000/0.000	1.000
	TGACCTCACAGATTGCATCC				*Cfu*	20/3	239–247	0.099/0.100	1.000
					Egu	17/10	254–280	0.840/0.706	0.298
*Mti*59	AACATAATGTGACGGTGTGATG^{HEX}	JX091820	$(TG)_{16}$	94	*Mve*	20/5	97–111	0.478/0.450	0.773
	CGGGTGTGATCTCTACATTTTTC				*Cfu*	20/4	94–100	0.691/0.600	0.023
					Egu	20/17	103–139	0.950/0.900	0.276
*Mti*60	CCAGTTTCCAAAGCCACAAC^{NED}	JX091821	$(TG)_{24}$	184	*Mve*	19/10	190–228	0.744/0.632	0.197
	CCTGTTGGAGATAAACACACAC				*Cfu*	20/4	170–180	0.583/0.300	**0.001**
					Egu	16/18	171–227	0.946/1.000	0.418
*Mti*61	CGTGAGAAACATCTCGTCTCC^{FAM}	JX091822	$(TG)_{11}$	199	*Mve*	20/2	205–207	0.050/0.050	1.000
	TGCTGTAACACCAGAAATGAAAC				*Cfu*	20/18	201–253	0.914/0.900	0.470
					Egu	20/7	229–249	0.488/0.450	0.630

[a] Primer sequences are forward (top) and reverse (bottom); [b] GenBank Accession number; [c] Repeat motif; [d] Size (in base pairs) of the allele in the sequenced clone; [e] Species characterized: yellowfin grouper (*Mve*), coney (*Cfu*), and red hind (*Egu*); [f] N is number of individuals assayed, N_A is number of alleles identified; [g] Size range for alleles detected (includes the 21 bp 5′-tail-sequence); [h] H_E is expected heterozygosity, H_O is observed heterozygosity; [i] P_{HW} is probability of deviation from Hardy–Weinberg expectations—significant deviations are noted in bold. The fluorescent 5′-tail-sequence label attached to the forward (top) primer is noted as 6-FAM^{FAM}, NED^{NED}, or HEX^{HEX}. Species in which a given marker failed to amplify are indicated by X

of ampicillin and 8 % glycerol), and incubated at 37 °C overnight to increase culture density. For each microsatellite enrichment (dinucleotide and trinucleotide), a single tissue culture plate was then sent to the Interdisciplinary Center for Biotechnology Research at the University of Florida (http://www.biotech.ufl.edu/) for sequencing with the M13 forward primer. Resulting sequences were edited and vector trimmed with Sequencher 4.1 (Gene Codes); clones containing viable microsatellite motifs were identified with Simple Sequence Repeat Identification Tool (SSRIT, http://www.graene.org/db/markers/ssrtool); primer pairs were developed using Primer3 (http://frodo.wi.mit.edu/).

A total of 61 unlabeled primer pairs (34 from the dinucleotide enrichment plate and 27 from the trinucleotide enrichment plate) were purchased from Integrated DNA Technologies (IDT); the forward primer included a 21-bp 'tail' sequence (5′-GCCTCGTTTATCAGATGTGGA-3′), allowing for the simultaneous amplification and fluorescent labeling of targeted microsatellite fragments (Karlsson et al. 2008). The 5′-tail-sequence oligonucleotide was labeled with one of three fluorescent dyes: 6-Fam, Hex, or Ned (Set D, Applied Biosystems). The original intent of the project was to characterize the markers for the species of origin, *M. tigris*. General difficulties in obtaining tiger groupers and layoffs at Caribbean resource management agencies normally responsible for monitoring tiger grouper spawning aggregations precluded procuring a sufficient number of samples. The markers were instead characterized in the congeneric species, *Mycteroperca venenosa* (yellowfin grouper) and two related epinepheline species, *Cephapholis fulva* (coney) and *Epinephelus guttatus* (red hind). Each primer pair was evaluated initially with a subset of twelve individuals, four from each of the three species. Successful amplifications were evaluated further with an additional sixteen individuals from each species (60 individuals total), all sampled from waters off the coast of St. Thomas (U.S. Virgin Islands). All DNA samples were extracted from fin clips, using a modified Chelex protocol (Estoup et al. 1996). PCR products were run on an ABI 377 DNA Sequencer; alleles were sized using the Genescan® 400HD [Rox] Size Standard (Applied Biosystems); allele sizing and calling were performed with Genescan® 3.1.2 and Genotyper® version 2.5 software. Genetic variability of the microsatellite markers was measured by the number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity. Fisher's exact tests, as implemented in GDA (Lewis and Zaykin 2001), were used to test significance of departure from Hardy–Weinberg equilibrium (genotype) expectations at individual microsatellites and of departure from genotypic equilibrium at pairs of microsatellites. Evidence for occurrences of null alleles was explored using Microchecker (van Oosterhout et al. 2004).

Of the initial 61 putative microsatellites identified, 31 primer pairs produced easily scored polymorphic PCR amplifications in *M. venenosa* (Table 1). The number of alleles detected ranged from two (*Mti*22, *Mti*23, *Mti*61) to 20 (*Mti*39); expected heterozygosity ranged from 0.050 (*Mti*61) to 0.962 (*Mti*39), while observed heterozygosity ranged from 0.050 (*Mti*61) to 1.000 (*Mti*51). All individual microsatellites and microsatellite pairs conformed to Hardy–Weinberg expectations and genotypic equilibrium, respectively, following Bonferroni correction for multiple tests (Rice 1989). Analysis with Microchecker indicated no evidence for null alleles at any of the 31 microsatellites.

A total of 17 primer pairs produced easily scored polymorphic PCR amplifications in *C. fulva* (Table 1). The number of alleles ranged from 2 (*Mti*16, *Mti*31) to 25 (*Mti*35); expected heterozygosity ranged from 0.050 (*Mti*16, *Mti*31) to 0.977 (*Mti*35), while observed heterozygosity ranged from 0.050 (*Mti*16, *Mti*31) to 0.950 (*Mti*24, *Mti*35, *Mti*39). Genotypes at one microsatellite (*Mti*60) deviated significantly from Hardy–Weinberg expectations following Bonferroni correction for multiple tests, and two pairs of microsatellites (*Mti*60/*Mti*16 and*Mti*60/*Mti*43) deviated significantly from genotypic equilibrium following Bonferroni correction. Analysis with Microchecker indicated evidence for null alleles at *Mti*60, along with possible scoring errors due to stuttering.

A total of 23 primer pairs produced easily scored polymorphic PCR amplifications in *E. guttatus* (Table 1). The number of alleles ranged from 2 (*Mti*16, *Mti*22) to 22 (*Mti*31); expected heterozygosity ranged from 0.104 (*Mti*42) to 0.962 (*Mti*34), while observed heterozygosity ranged from 0.053 (*Mti*42) to 1.000 (*Mti*34, *Mti*39, *Mti*60). Genotypes at two microsatellites (*Mti*10, *Mti*12) deviated significantly from Hardy–Weinberg expectations following Bonferroni correction for multiple tests, and five pairs of microsatellites (*Mti*10/*Mti*12, *Mti*10/*Mti*43, *Mti*10/*Mti*53, *Mti*12/*Mti*25, and *Mti*12/*Mti*43) deviated significantly from genotypic equilibrium following Bonferroni correction. Analysis with Microchecker indicated evidence for null alleles at both *Mti*10 and *Mti*12.

The microsatellite markers identified were isolated from genomic DNA of tiger grouper, *M. tigris*, and were used to cross-amplify in one congener and two species in the same subfamily. A total of 36 microsatellites appear to be useful for future research with members of the subfamily Epinephelinae.

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