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TECHNICAL NOTE

Isolation and characterization of microsatellite markers from yellowfin grouper, *Mycteroperca venenosa*

Mark A. Renshaw · Richard S. Nemeth · John R. Gold

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Abstract Twenty-seven nuclear-encoded microsatellites were isolated from an enriched genomic library of yellowfin grouper, *Mycteroperca venenosa,* and characterized in 24 individuals. The microsatellites include 17 dinucleotide repeats, eight trinucleotide repeats (one imperfect), one tetranucleotide repeat, and one pentanucleotide repeat. Yellowfin grouper are susceptible to fishing pressure targeted towards their spawning aggregations; data pertaining to stock structure and levels of genetic variability will aid in future management of this species.

Keywords Microsatellites · Yellowfin grouper · *Mycteroperca venenosa*

The yellowfin grouper, *Mycteroperca venenosa*, is one of the most abundant groupers in the Caribbean, ranging from the Gulf of Mexico southward to Brazil (Heemstra and Randall 1993). Similar to other members of the family Serranidae, yellowfin grouper form large, annual spawning aggregations that are susceptible to targeted fishing pressure (Nemeth et al. 2006). The species is currently listed as 'Near Threatened' (Brule and Garcia-Moliner 2004) by the IUCN (International Union for Conservation of Nature and Natural Resources), with the additional note that declining trends in both the landings and sizes of spawning aggregations indicate that *M. venenosa* is nearing a 'Vulnerable' listing (SEDAR14 2007).

Management of an exploited fishery can benefit from stock-structure assessments, both within and across political boundaries. Nuclear-encoded microsatellite markers are well suited for population-genetic analysis, including identification of stocks and assessment of genetic variability within and between spawning aggregations (Ward 2000). Here, we report the development of polymerase chain reaction (PCR) primers for 27 microsatellites from an enriched yellowfin grouper genomic DNA library.

Details for the generation of the enriched microsatellite library can be found in Renshaw et al. (2010). Ten microliters of the size-selected genomic DNA/linker fragments were hybridized in each of three independent 100 µl reactions (6× SSC [0.9 M NaCl, 0.09 M sodium citrate] final concentration): one reaction employed 50 pmol of 3′-biotin-modified $(CA)_{13}$ oligonucleotides; a second reaction employed 50 pmol of 3′-biotin-modified $(CAT)_8$ oligonucleotides; while a third reaction employed 50 pmol of 3′-biotin-modified $(GAT)_8$ oligonucleotides. Hybridization mixtures were heated to 95°C for 10 min and then kept at 58°C [$(CA)_{13}$ hybridization] or 47°C [$(CAT)_8$ and $(GAT)_8$ hybridizations] for 1.25 h. Following the hybridization step, both trinucleotide reactions were combined for subsequent incubation with the streptavidin-coated magnetic beads; the dinucleotide reaction was incubated separately, producing independent dinucleotide and trinucleotide enrichments. Positive (white) colonies were picked with sterile toothpicks, placed in 96-well tissue culture plates with 200 µl LB broth (containing 50 µg/ml of ampicillin and 8% glycerol), and incubated at 37°C overnight to increase density of the cultures. Culture plates were then sent to the Interdisciplinary Center for Biotechnology Research at the University of Florida (http://www.biotech.ufl.edu/) for sequencing

M. A. Renshaw (✉) · J. R. Gold
Center for Biosystematics and Biodiversity, Texas A and M University, College Station, TX 77843-2258, USA
e-mail: mrenshaw@ag.tamu.edu

R. S. Nemeth
Center for Marine and Environmental Studies, University of the Virgin Islands, 2 John Brewer's Bay, St. Thomas, Virgin Islands 00802-9990, USA

Table 1 Summary data for 27 microsatellites characterized for the yellowfin grouper, *Mycteroperca venenosa*

Microsatellite	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone Size[d]	N/N_A^e	Size Range[f]	H_E/H_O^g	P_{HW}^h
*Mve*19	ACCCAAGTGGCTCTCTAACC^{HEX} ATGCAAGTCTAGCCGTCAGG	HQ395354	$(GT)_9$	136	21/2	157–159	0.215/0.238	1.0000
*Mve*21	CACTTAGCGGTTGATTGACG^{FAM} GGGATTAGAACCCACACAGC	HQ395355	$(CA)_{26}$	242	20/10	237–269	0.860/0.750	0.4159
*Mve*24	ACGCCATGATGAGAGATGG^{NED} TCAGTGCATGCATGTTTTCC	HQ395356	$(AC)_9$	194	24/3	212–220	0.563/0.583	0.7716
*Mve*25	CACCAGTGTGTTGCAGAAGG^{FAM} CCCTGGCTCTCTACCAAGC	HQ395357	$(ACAG)_6$	236	23/2	252–256	0.085/0.087	1.000
*Mve*26	CTTCCTGCTTTACACACACACA^{FAM} ATGACGATGGACACACGAAA	HQ395358	$(CA)_{18}$	178	23/15	185–225	0.886/0.913	0.7819
*Mve*27	CTGTGTGAGCAGCGTAGAGG^{NED} TGTGGACGCTTTCTCTGTCC	HQ395359	$(TG)_7$	182	22/2	202–204	0.089/0.091	1.0000
*Mve*30	GGCTTACAAGCAGCACACG^{NED} GCTTTGCAGGGTGATGTAGG	HQ395360	$(AC)_{10}$	141	24/2	161–163	0.112/0.125	1.0000
*Mve*33	CCACACCTTCTTCGAGATGC^{HEX} TGTCCAGAGCGTCCTCTCC	HQ395361	$(CA)_{14}$	123	23/6	143–161	0.495/0.478	0.5563
*Mve*37	GTCTCACTGATCACCAACAGC^{HEX} TGACAGACAGAGGACGATGG	HQ395362	$(CAT)_{17}$-3 bp-$(CAT)_5$	114	23/11	92–149	0.861/0.696	0.1263
*Mve*38	CAGGTCCCACAGTCATTATCC^{FAM} AGAAGCCTCGACAGTCTTGC	HQ395363	$(CA)_{12}$	232	23/4	252–264	0.243/0.217	0.1863
*Mve*40	TGTGGCTCTGACACACACG^{NED} CAAAATGTTCCTGTGGTTTGG	HQ395364	$(CA)_{25}$	166	23/18	173–221	0.936/0.957	0.6497
*Mve*42	TCCAGCCCTCATTAGCAAAC^{FAM} CCCTGTGTCCGTGTGTGTG	HQ395365	$(CA)_{15}$	253	20/7	261–277	0.800/0.850	0.8241
*Mve*43	CAGTGAGTCGGGATACACAGC^{FAM} GGAATGACTCCTAAAAAGACAGG	HQ395366	$(TCA)_7$	219	23/4	234–246	0.532/0.565	0.7197
*Mve*44	ACGCACTGCACACACATATC^{HEX} GACACAGCAGAGCAGCAGAC	HQ395367	$(CA)_{11}$	114	24/6	126–142	0.735/0.708	0.6578
*Mve*45	CCAGCACACACAAATGAAGG^{FAM} TGTAGCGGAAAAGATGTCTGC	HQ395368	$(CA)_{17}$	281	22/4	294–322	0.214/0.227	1.0000
*Mve*47	TTTCAGATCGTCACACAGAATGTG^{HEX} GATTCGATCATCATCATCATCATC	HQ395369	$(GAT)_{12}$	107	21/5	117–132	0.405/0.476	1.0000
*Mve*52	ACAGGTTGGGAGGAGATGG^{HEX} GAAAGAGCCCATCAGAAACG	HQ395370	$(GAT)_{10}$	175	24/7	181–202	0.789/0.792	0.9997

Table 1 continued

Microsatellite	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone Size[d]	N/N_A^e	Size Range[f]	H_E/H_O^g	P_{HW}^h
*Mve*53	GCCCTGTTCTTCAGAACTCC**HEX** ATGGGGATCAAACACACAGC	HQ395371	$(AC)_{10}$	97	24/2	117–121	0.082/0.083	1.0000
*Mve*57	TCTGACCTTGACGACAGACG**HEX** TGGAAGGTTTTGACGTTTCC	HQ395372	$(AC)_{15}$	130	24/9	147–169	0.772/0.833	0.4672
*Mve*61	AGGGCATTCGTTGTTTTCC**HEX** GGACCTTCACAACTGATAGGG	HQ395373	$(CAT)_8$	147	24/3	162–168	0.630/0.583	0.4425
*Mve*63	ATCATTGCTCTCTGCTGACG**NED** TTTAGTCAAGTGAACCCTCATCC	HQ395374	$(TGA)_6$	202	23/4	224–233	0.622/0.522	0.4463
*Mve*64	CCAAAGCACCACTTTGTCC**NED** GGCAGTGACTCCCTTAGGC	HQ395375	$(GT)_{26}$	192	24/12	199–229	0.893/1.000	0.6113
*Mve*68	AGAAAGCAGTGCAAACATGC**HEX** TCTCCCAGTCCTTCATTTGG	HQ395376	$(AC)_{26}$	144	24/14	136–182	0.890/0.792	0.3559
*Mve*70	ACAGTAGCCTCGGCCATCAG**FAM** GGAGTGGCTGTGATCAGCTC	HQ395377	$(TG)_{16}$	221	24/5	237–247	0.695/0.708	0.7959
*Mve*71	TCGATTCAAACAGCTGCAAG**FAM** ACACGTTCGCTTCATGTGTG	HQ395380	$(TGA)_{14}$	206	24/5	213–228	0.621/0.500	0.0075
*Mve*74	CACAGGGACATGTTGGACAG**HEX** AGCCAATCAAAACAGCCAAG	HQ395378	$(GAT)_{18}$	131	24/11	124–202	0.715/0.500	0.0053
*Mve*75	TCAGAGAGACGAACCAGCAC**FAM** CAAACAGCAGCAGACGAGAG	HQ395379	$(ACGTC)_5$	300	23/6	316–341	0.725/0.739	0.6416

[a] Primer sequences are forward (top) and reverse (bottom); [b] GenBank Accession number; [c] Repeat indicates repeat motif; [d] Clone Size is the size (in base pairs) of the allele in the sequenced clone; [e] N is the number of individuals assayed, and N_A is the number of alleles detected; [f] Size Range refers to alleles thus far uncovered (includes the 21 bp 5′-tail-sequence); [g] H_E and H_O are expected and observed heterozygosity, respectively; [h] P_{HW} represents the probability of deviation from Hardy–Weinberg expectations. The fluorescent 5′-tail-sequence label attached to the forward (top) primer was either 6-FAM**FAM**, HEX**HEX**, or NED**NED**

with M13 primers. Resulting sequences were edited and vector trimmed with SEQUENCHER 4.1 (Gene Codes); primer pairs were developed using PRIMER3 (http://frodo.wi.mit.edu/).

Unlabelled primers were purchased from Integrated DNA Technologies (IDT); the forward primer included a 21 bp sequence (5′-GCCTCGTTTATCAGATGTGGA-3′) that enabled amplified fragments to be labeled fluorescently during PCR amplifications (Karlsson et al. 2008). The 5′-tail-sequence primer was labeled with one of three fluorescent labels: 6-FAM, HEX, or NED (Set D, Applied Biosystems). Each primer pair was initially evaluated with a subset of nine individuals; successful amplifications were evaluated further with an additional 15 individuals (24 individuals total), all sampled from waters off St. Thomas (US Virgin Islands). PCR products were run on an ABI 377 automated sequencer. Alleles were sized using the GENESCAN®-400 HD ROX Size Standard (Applied Biosystems); allele sizing and calling were performed using GENESCAN® 3.1.2 and GENOTYPER® version 2.5 software. Genetic variability of the microsatellite markers was measured by number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity. Fisher's exact test, as implemented in GDA (Lewis and Zaykin 2001), was used to test significance of departure from Hardy–Weinberg equilibrium (genotype) expectations at each microsatellite 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 75 putative microsatellites identified, 27 PCR primer pairs produced experimentally tractable microsatellites. Summary data for these 27 microsatellites are presented in Table 1. The number of alleles detected ranged from two (*Mve*19, *Mve*25, *Mve*27, *Mve*30, *Mve*53) to 18 (*Mve*40); expected heterozygosity ranged from 0.082 (*Mve*53) to 0.936 (*Mve*40), while observed heterozygosity ranged from 0.083 (*Mve*53) to 1.000 (*Mve*64); genotypes at all microsatellites conformed to Hardy–Weinberg expectations following Bonferroni correction (Rice 1989). Analysis using MICROCHECKER indicated no evidence for scoring error due to stuttering or large allele dropout at any of the 27 microsatellites; possible null alleles were suggested only at *Mve*74. None of the pair-wise comparisons of microsatellites deviated significantly from genotypic equilibrium following Bonferroni corrections (Rice 1989).

The 27 microsatellites characterized in this study will prove useful for future population-genetic research in *Mycteroperca venenosa* as well as other members of the family Serranidae.

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