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PRIMER NOTE

Isolation and characterization of microsatellites in lane snapper (*Lutjanus synagris*), mutton snapper (*Lutjanus analis*), and yellowtail snapper (*Ocyurus chrysurus*)

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Abstract

Polymerase chain reaction primer pairs for a total of 25 nuclear-encoded microsatellites (loci) were developed from genomic DNA libraries of lane snapper (*Lutjanus synagris*), mutton snapper (*Lutjanus analis*), and yellowtail snapper (*Ocyurus chrysurus*). The microsatellites include 24 perfect (21 dinucleotide and three trinucleotide) and one imperfect (combination tetranucleotide/tetranucleotide) repeat motifs. A total of 32 individuals of each species were assayed for allelic variation at all 25 microsatellites; reliable amplification products were generated for lane snapper (25 loci), mutton snapper (21 loci), and yellowtail snapper (24 loci). Significant deviations from Hardy–Weinberg expectations, following Bonferroni corrections, were found for one microsatellite in lane and yellowtail snappers, and for three microsatellites in mutton snapper. All pairwise comparisons of microsatellites (all three species) did not deviate significantly from genotypic equilibrium.

Keywords: *Lutjanus analis*, *Lutjanus synagris*, microsatellites, *Ocyurus chrysurus*

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Lane snapper, *Lutjanus synagris*, mutton snapper, *Lutjanus analis*, and yellowtail snapper, *Ocyurus chrysurus*, are reef-associated lutjanids distributed in the western Atlantic along the southeastern USA southward to Brazil (Manooch & Mason 1984; Manooch & Drennon 1987; Burton 2002). All three species are important components of commercial and recreational fisheries in US waters, including the greater Caribbean region (Manooch & Mason 1984; Manooch & Drennon 1987; Burton 2002). In this study, we report development of polymerase chain reaction (PCR) primers for a total of 25 nuclear-encoded microsatellites developed from genomic DNA libraries of all three species. Nuclear-encoded microsatellites are well suited for providing information on population-genetic structure and effective population size, parameters of paramount interest to management of marine fishery resources (Saillant & Gold 2006).

For each of the three species, whole genomic DNA was extracted from ethanol-preserved muscle tissue, using a standard phenol–chloroform method, and digested with *Dpn*II (New England BioLabs). A total of 18 432 clones (48 × 384-well plates), 6144 from each of the three species (16 × 384-well plates), were generated as described in Renshaw *et al*. (2006) and spotted in a 4 × 4 array onto 22.5 × 22.5 cm Hybond-N+ nylon membranes (Amersham), with each clone being spotted twice to eliminate false positives. Clones were fixed on membranes and probed two different times with a variety of (gamma) ^{32}P-labelled oligonucleotides: (i) one tetranucleotide/trinucleotide cocktail with $(GATA)_9$, $(CATA)_8$, $(GACA)_8$, $(CAA)_8$, $(GAA)_8$, and $(TAA)_{13}$, and (ii) one dinucleotide cocktail with $(CA)_{13}$ and $(GA)_{13}$. A total of 30, 47, and 40 positive clones, respectively, were screened from the lane snapper (*Lsy*), mutton snapper (*Lan*), and yellowtail snapper (*Och*) libraries. Plasmid DNA was amplified using the 555 and 837 primers of Makova & Patton (1998) and visualized on a 2% agarose gel; target DNA fragments were selected by extraction and purified using a gel extraction kit (QIAGEN). Purified DNA was quantified and both strands sequenced using the 555 and 837 primers and ABI PRISM BigDye Terminator version 3.1 (Applied Biosystems). Products were electrophoresed on an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems). SEQUENCHER 3.0 (Gene

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Codes) was used for DNA base-call editing and vector trimming. A total of 12, 14, and 10 primer pairs for *Lsy*, *Lan*, and *Och*, respectively, were developed using AMPLIFY 1.2 (Engels 1993) and NETPRIMER (http://www.premierbiosoft.com/netprimer).

Unlabelled PCR primers were purchased from Invitrogen and tested for amplification by screening two individuals of each species. PCR amplifications were performed with a PTC-200 thermocycler (MJ Research) in 10 μL reaction volumes containing 100 ng DNA, 1 × PCR buffer (50 mM KCl, 10 mM Tris, 1% Triton-X 100), 0.1 U *Taq* DNA polymerase (Invitrogen), 0.5 μM of each primer, 200 μM of each dNTP, and 1 mM $MgCl_2$. PCR conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 38 cycles of denaturation at 95 °C for 30 s, annealing at 45–65 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. Once appropriate annealing temperatures were established for each primer pair, microsatellite arrays were tested for polymorphisms with an additional four individuals of each species (= six individuals per species tested). Based on successful amplifications in all three species, a total of 10 (*Lsy*), seven (*Lan*), and eight (*Och*) microsatellites were chosen for further screening (Table 1). The 25 (total) microsatellites consisted of 24 perfect (21 dinucleotide and three trinucleotide) and one imperfect (combination tetranucleotide/tetranucleotide) repeat motifs. Length of cloned alleles in the species of origin ranged in size from 80 to 323 base pairs; optimal annealing temperatures ranged from 48 to 58 °C (Table 1).

Table 1 Summary information for microsatellite markers developed from lane (*Lsy*), mutton (*Lan*), and yellowtail (*Och*) snappers

Locus	GenBank Accession no.	Primer sequence (5′–3′)	Repeat sequence	T_a (°C)	ABI dye	Clone size
Lsy 1	EF204579	**CGACCAACACATTCAAACT** GACATCAACACTATGACAGGC	$(CA)_{18}$	52	NED	226
Lsy 4	EF204580	**CCTGTGGTTTGCTGATAG** GCATTAGTTGTTGCTGTCAC	$(CA)_{32}$	52	HEX	242
Lsy 5	EF204581	**CCAAGTTGATGCTTTGATTCTC** CCTGAAAAAGGAGAGACACGG	$(CTT)_{24}$	48	HEX	186
Lsy 6	EF204582	**GGAAAGAGAAGGACGGAGGA** GCTGCTACCACTGACGAGAAT	$(CA)_{18}$	58	HEX	257
Lsy 7	EF204583	**GCTGTAATCAAATCCCTGTG** GGTTCTCCAACTGTTCTCCT	$(CA)_{12}$	58	NED	196
Lsy 8	EF204584	**GCTGCTGCTTCACTGGA** GGAAACGAGTTGTTGTGACG	$(CA)_{17}$	58	NED	240
Lsy 10	EF204585	**GTCAGCGTTGCTCTATCAGTG** GGAGAGATGGTTCCCTCAGT	$(CA)_{23}$	55	FAM	129
Lsy 11	EF204586	**GACATTGTAACACTTGGTCAC** CCCTATTGAATGTAAGTGAGAC	$(CA)_{28}$	55	FAM	239
Lsy 13	EF204587	**GCTGCACAGTGTGTTACCAG** GCTGAAGGAAGATTTGGAC	$(CA)_{15}$	58	NED	139
Lsy 14	EF204588	**CCTCTTCCACCACATTATTCTC** CCAGTATGTTTGAAAGGCG	$(CA)_{12}$	58	FAM	172
Lan 3	EF204564	**CAGAGAGACACATCACTCAGACAC** GCTCCTGAGTTGGAACAGTG	$(CAA)_{11}$	48	FAM	150
Lan 5	EF204565	**CCGTCTTACTTCAGCATTTG** GGCTTTTGTAGTGTTTCCA	$(GACA)_4(GATA)_{15}$	52	FAM	173
Lan 6	EF204566	**CCCAAATGATTCCTCTGAGTGTC** GGTACTTGTGTGTGAGCGTGTAGTT	$(CA)_{12}$	58	FAM	242
Lan 9	EF204567	**GCTTCACACTCGCTGACACAT** GAGCCATTCTTCAACTCAACATC	$(CA)_{11}$	58	HEX	236
Lan 11	EF204568	**CCACAGAGTCCAAAGCAGAAAG** GCATCCACACACAGTAATCAGG	$(CA)_{22}$	58	NED	267
Lan 12	EF204569	**CCTCCAACATCTGACTCAAAGC** GGTGAGAGTGTTTGTGCGAATG	$(CA)_{10}$	58	NED	207
Lan 13	EF204570	**GCAGTCCTCTTTGAGTCCA** CTGCTTGACTGAGCGATAA	$(CA)_{10}$	55	HEX	160
Och 2	EF204571	**GGACAGTATCACTATTCTCGC** CCACAAGGTGTTGCTACTAA	$(CA)_{18}$	50	NED	147

Table 1 *Continued*

Locus	GenBank Accession no.	Primer sequence (5′–3′)	Repeat sequence	T_a (°C)	ABI dye	Clone size
Och 4	EF204572	**CGTCACTATGTGTCGCTAATCCGTT** GGCTCATTTCTTCAGTCGTTTGG	$(CA)_{14}$	58	FAM	188
Och 6	EF204573	**CCTCTGGCATACATCTCACATC** GCACACAAACACACCTCACCT	$(CA)_{20}$	55	HEX	250
Och 9	EF204574	**GCTCGTTCACTCTTAACATCAAC** GCTGTCAGTGTCAAGGTGTATG	$(CA)_{14}$	58	HEX	78
Och 10	EF204575	**CTCAGACAGTGGTTTAACAGGATG** CAGCATAGAGAACAATGTCAGTCA	$(GGA)_{11}$	58	FAM	321
Och 11	EF204576	**CCAGATACACTGATGCTAACCA** GGAGATGCCACGCTGC	$(CA)_{28}$	58	NED	127
Och 13	EF204577	**CCTCATGCTTCAAACACACG** CTCTTCATCCCAAAACACAG	$(CA)_{13}$	55	FAM	91
Och 14	EF204578	**GGAGGTGTTGACAGCACA** CCTTGAAACCGTCCTGAT	$(GA)_{10}$	55	HEX	140

GenBank Accession numbers for clone sequences; Primer sequences are forward (top in bold) and reverse (bottom); Repeat sequence indicates repeat motif; T_a is annealing temperature in °C; ABI dye is the fluorescent label attached to the forward primer; Clone size is the size of the clone allele in base pairs.

For further screening, the forward primer from each primer pair was labelled with one fluorescent label of Set D (Applied Biosystems: FAM, HEX, or NED) as indicated in Table 1. DNA was extracted from a total of 32 individuals of each species; lane snapper were obtained from Fort Myers, Florida, whereas mutton and yellowtail snappers were obtained from Key West, Florida. For each microsatellite, PCR products were amplified from all three species (total of 96 amplifications per locus) and run on an ABI 377 automated sequencer. Alleles were sized using the GENESCAN-400 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 each microsatellite was evaluated as the number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity. Fisher's exact test, as implemented in GDA (Lewis & Zaykin 2001), was used to test for departure from Hardy–Weinberg equilibrium (genotype) expectations at each microsatellite and for departure from genotypic equilibrium at pairs of microsatellites. Evidence for the presence of null alleles at each locus was evaluated with MICRO-CHECKER (Van Oosterhout *et al*. 2004).

Summary genotypic data are presented in Table 2. All 25 primer pairs produced easily scored amplification products in lane snapper, and following Bonferroni correction (Rice 1989), genotypes at all but one locus (*Och* 6) conformed to Hardy–Weinberg expectations. None of the pairwise comparisons of microsatellites deviated significantly from genotypic equilibrium, but support for the presence of null alleles at two loci (*Lan* 5 and *Och* 6) was indicated by MICRO-CHECKER.

Four primer pairs (for microsatellites *Lsy* 6, *Lsy* 7, *Och* 2, and *Och* 13) did not generate easily scored amplification products in mutton snapper, and genotypes at three microsatellites (*Lsy* 10, *Lsy* 14, and *Och* 11) deviated significantly from Hardy–Weinberg expectations. None of the pairwise comparisons of microsatellites deviated significantly from genotypic equilibrium, but support for the presence of null alleles at three microsatellites (*Lsy* 10, *Lsy* 14, and *Och* 6) was indicated by MICRO-CHECKER.

For yellowtail snapper, one primer pair (for microsatellite *Lsy* 10) did not generate easily scored amplification products, and one microsatellite (*Och* 14) showed significant deviation from Hardy–Weinberg expectation. None of the pairwise comparisons of microsatellites deviated significantly from genotypic equilibrium, but support for the presence of null alleles at four microsatellites (*Lan* 3, *Lan* 9, *Och* 4, and *Och* 14) was indicated by MICRO-CHECKER. The 25 microsatellites developed in this study will prove useful for providing information on population-genetic structure and effective population size for these three commercially and recreationally exploited snappers.

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Table 2 Summary genotypic data from 32 individuals each for lane (*Lsy*), mutton (*Lan*), and yellowtail (*Och*) snappers

	N/N_a			Size range			H_E			H_O			P_{HW}		
Msat	*Lsy*	*Lan*	*Och*	*Lsy*	*Lan*	*Och*	*Lsy*	*Lan*	*Och*	*Lsy*	*Lan*	*Och*	*Lsy*	*Lan*	*Och*
Lsy 1	28/14	29/12	29/2	212–268	212–262	201–203	0.844	0.889	0.390	0.821	0.828	0.448	0.178	0.178	0.634
Lsy 4	31/28	32/4	29/2	205–281	188–194	192–194	0.967	0.258	0.034	1.000	0.250	0.034	1.000	0.337	1.000
Lsy 5	27/13	31/5	29/14	137–191	149–182	155–200	0.786	0.364	0.893	0.630	0.387	0.862	0.019	0.478	0.608
Lsy 6	30/16	**X**	29/3	253–286	**X**	244–248	0.902	**X**	0.520	0.867	**X**	0.621	0.303	**X**	0.429
Lsy 7	31/7	**X**	29/20	190–220	**X**	243–305	0.703	**X**	0.944	0.645	**X**	0.931	0.429	**X**	0.809
Lsy 8	31/12	32/1	29/3	222–272	228	242–246	0.643	0.000	0.163	0.710	0.000	0.103	0.813	1.000	0.167
Lsy 10	31/21	32/15	**X**	119–171	113–147	**X**	0.946	0.912	**X**	1.000	0.688	**X**	0.953	0.000	**X**
Lsy 11	31/20	32/13	29/7	218–266	215–251	212–244	0.928	0.771	0.700	0.968	0.844	0.724	0.984	0.268	0.693
Lsy 13	31/14	32/6	29/13	127–159	125–145	133–159	0.779	0.764	0.857	0.839	0.750	0.793	0.869	0.749	0.030
Lsy 14	31/6	30/5	28/2	164–178	158–170	156–158	0.455	0.480	0.135	0.452	0.200	0.143	0.187	0.000	1.000
Lan 3	29/10	31/10	29/9	137–164	143–173	127–154	0.862	0.843	0.848	0.828	0.871	0.690	0.927	0.906	0.008
Lan 5	25/15	29/31	29/20	168–240	150–300	161–289	0.938	0.973	0.941	0.680	1.000	0.862	0.003	0.773	0.258
Lan 6	30/7	30/15	29/5	237–259	233–271	253–261	0.635	0.909	0.329	0.600	1.000	0.241	0.591	0.293	0.265
Lan 9	26/1	30/10	29/10	226	230–250	228–248	0.000	0.821	0.828	0.000	0.800	0.655	1.000	0.722	0.115
Lan 11	30/10	27/17	29/11	249–289	246–292	230–272	0.717	0.892	0.808	0.667	0.926	0.931	0.569	0.106	0.509
Lan 12	31/3	32/4	29/4	202–208	198–208	206–218	0.154	0.678	0.571	0.161	0.594	0.621	1.000	0.033	0.839
Lan 13	31/6	32/3	29/15	157–167	156–160	183–233	0.597	0.325	0.828	0.548	0.344	0.724	0.082	0.719	0.063
Och 2	31/18	**X**	29/16	140–192	**X**	140–192	0.913	**X**	0.906	0.806	**X**	0.987	0.023	**X**	0.789
Och 4	31/5	32/4	29/6	183–193	177–185	183–197	0.645	0.550	0.805	0.645	0.531	0.621	0.883	0.657	0.189
Och 6	27/6	30/17	29/11	242–252	226–280	229–259	0.530	0.912	0.860	0.259	0.733	0.793	0.000	0.010	0.096
Och 9	30/7	32/13	29/9	57–79	55–83	64–84	0.725	0.878	0.634	0.800	0.844	0.517	0.410	0.550	0.189
Och 10	30/1	32/1	29/5	299	298	310–331	0.000	0.000	0.520	0.000	0.000	0.414	1.000	1.000	0.184
Och 11	31/15	32/20	28/14	95–147	123–157	92–138	0.885	0.942	0.871	0.903	0.844	0.857	0.498	0.001	0.830
Och 13	31/5	**X**	29/9	79–91	**X**	81–105	0.365	**X**	0.811	0.290	**X**	0.828	0.022	**X**	0.479
Och 14	30/2	30/1	29/5	138–140	136	130–140	0.033	0.000	0.716	0.033	0.000	0.310	1.000	1.000	0.000

Msat, microsatellite; N is the number of amplified individuals and N_a is the number of alleles detected; Size range refers to alleles thus far uncovered (in base pairs); H_E and H_O are expected and observed heterozygosities, respectively; P_{HW} represents the probability of conforming to Hardy–Weinberg expectations. Significant deviations from Hardy–Weinberg expectations, following Bonferroni correction (Rice 1989), are underlined. Loci that were not scoreable for a given species are marked **X** within the table.

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