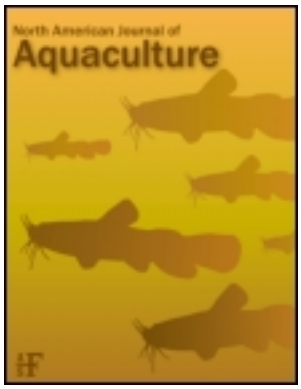

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Characterization of Greater Amberjack Microsatellite Markers in Lesser Amberjacks, Yellowtail Jacks, Almaco Jacks, and Banded Rudderfish

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TECHNICAL NOTE

Characterization of Greater Amberjack Microsatellite Markers in Lesser Amberjacks, Yellowtail Jacks, Almaco Jacks, and Banded Rudderfish

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Abstract

Thirty-one microsatellite markers that were previously isolated from and characterized in greater amberjacks *Seriola dumerili* were assayed for cross-species amplification in four other members of the carangid genus *Seriola*: the lesser amberjack *S. fasciata*, yellowtail jack *S. lalandi*, almaco jack *S. rivoliana*, and banded rudderfish *S. zonata*. The number of markers that consistently amplified and were polymorphic ranged from 16 in yellowtail jacks to 25 in lesser amberjacks. The microsatellites characterized in this study will be useful for a variety of applications, including stock structure assessments of wild fish and parentage assignments of farmed fish.

Five species of the carangid genus *Seriola* support commercial and recreational fisheries along the coast of the mainland United States: the greater amberjack *S. dumerili*, lesser amberjack *S. fasciata*, yellowtail jack *S. lalandi*, almaco jack *S. rivoliana*, and banded rudderfish *S. zonata*. Three of the species (greater amberjack, yellowtail jack, and almaco jack) also are important components of commercial aquaculture production. The commercial aquaculture of greater amberjacks and yellowtail jacks is currently being researched or is already in progress in Japan, Australia, New Zealand, and a number of Mediterranean countries, including Italy, Spain, Malta, and Greece (Repulles-Albelda et al. 2008; Hamasaki et al. 2009; Stuart and Drawbridge, in press). In the United States, almaco jacks are produced commercially in Hawaii by using offshore aquaculture cages (Simpson 2011), and the production of yellowtail jacks is currently under research in California by utilizing broodfish that are collected locally from the wild (Stuart and Drawbridge, in press).

Nuclear-encoded microsatellites are useful markers for elucidating stock structure of "wild" fish (Miller et al. 2011) and can contribute to fisheries management decisions (Reiss et al. 2009). Microsatellites also are valuable for use in aquaculture programs, as they can serve as tools for parentage assignments (Gold et al. 2010), species authentication (Iguchi et al. 2012), and marker-assisted selection in "farmed" fish (Liu and Cordes 2004). In this paper, we evaluate the amplification of 31 microsatellites developed from the genomic DNA of greater amberjacks for cross-amplification in lesser amberjacks, yellowtail jacks, almaco jacks, and banded rudderfish.

METHODS

Ninety-six fish were assayed in this study: 19 lesser amberjacks (all from John's Island, South Carolina), 29 yellowtail jacks (all from San Diego, California), 30 almaco jacks (all from John's Island), and 18 banded rudderfish (7 from John's Island; 11 from Panama City, Florida). Fin clips were taken and placed in a 95% solution of ethanol (Florida

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samples), sarkosyl–urea (South Carolina samples), or a 20% solution of DMSO (California samples). Genomic DNA was extracted using a standard phenyl–chloroform protocol. The PCR primer sequences followed those given by Renshaw et al. (2006, 2007) for microsatellites isolated from the DNA of greater amberjacks. Unlabeled and fluorescently labeled primers (6-FAM and HEX) were obtained from Integrated DNA Technologies; primers that were fluorescently labeled with NED were obtained from Applied Biosystems, Inc. (ABI). The fluorescent label was attached to one of the primers from each microsatellite marker pair (Table 1).

The PCR amplifications were performed in 10-μL reactions by using 1 μL of DNA, 1 × Colorless GoTaq Flexi Buffer (Promega), 2-mM $MgCl_2$, 200 μm of each dNTP, 5 pmol of each primer (forward and reverse), and 0.5 unit of GoTaq Flexi DNA Polymerase (Promega). Cycling conditions employed (1) an initial denaturation step at 95°C for 3 min; (2) seven cycles with denaturation at 95°C for 30 s, first annealing temperature (defined below) for 45 s, and extension at 72°C for 1 min; (3) seven cycles with denaturation at 95°C for 30 s, second annealing temperature for 45 s, and extension at 72°C for 1 min; (4) 38 cycles with denaturation at 95°C for 30 s, third annealing temperature for 45 s, and extension at 72°C for 1 min; and (5) a final extension step at 72°C for 10 min. The first annealing temperature was the one listed by Renshaw et al. (2006, 2007), the second annealing temperature was 2°C lower than the first annealing temperature, and the third annealing temperature was 4°C lower than the first annealing temperature (Table 1). Amplified PCR products were run on an ABI 377 Automated Sequencer. Allele sizes were estimated with the GeneScan 400HD ROX Size Standard (ABI); allele sizes were determined using GeneScan version 3.1.2 and Genotyper version 2.5 (ABI). Genetic variability of each microsatellite marker was measured by the number of alleles, gene diversity (expected heterozygosity H_E), and observed heterozygosity (H_O) as calculated in Genetic Data Analysis (GDA) software (Lewis and Zaykin 2001). Fisher's exact tests as implemented in GDA were used to test for significant departures from Hardy–Weinberg expectations at individual microsatellites and for departures from genotypic equilibrium at pairs of microsatellites; Bonferroni corrections for multiple tests (Rice 1989) were applied manually to the *P*-value outputs from GDA. Micro-Checker software (Van Oosterhout et al. 2004) was used to test for evidence of null alleles, scoring errors due to stuttering, and scoring errors due to large-allele dropout at each microsatellite marker.

RESULTS AND DISCUSSION

Of the 31 microsatellites that were assayed, only *Sdu23* did not consistently amplify for at least one of the four species. Summary data for the remaining 30 microsatellites are presented in Table 1; previously published data for greater amberjacks (Renshaw et al. 2006, 2007) are also included in Table 1 to facilitate comparisons across species. In total, 25 markers were polymorphic for lesser amberjacks, and the number of alleles ranged from 2 to 16; H_E ranged from 0.053 to 0.930, while H_O ranged from 0.053 to 0.947. After Bonferroni correction for multiple tests (Rice 1989), genotypes at all markers conformed to Hardy–Weinberg expectations and all pairs of microsatellites were in genotypic equilibrium; analysis with Micro-Checker indicated no evident issues.

Sixteen markers were polymorphic for yellowtail jacks, with the number of alleles ranging from 2 to 14. The H_E ranged from 0.100 to 0.859, and H_O ranged from 0.103 to 1.000. After Bonferroni correction, genotypes at two markers (*Sdu29* and *Sdu46*) deviated significantly from Hardy–Weinberg expectations and 17 microsatellite pairs deviated significantly from genotypic equilibrium (*Sdu29–Sdu1*, *Sdu29–Sdu2*, *Sdu29–Sdu4*, *Sdu29–Sdu10*, *Sdu29–Sdu19*, *Sdu29–Sdu21*, *Sdu29–Sdu31*, *Sdu29–Sdu32*, *Sdu29–Sdu33*, *Sdu29–Sdu37*, *Sdu29–Sdu39*, *Sdu29–Sdu40*, *Sdu29–Sdu43*, *Sdu29–Sdu44*, *Sdu29–Sdu46*, *Sdu46–Sdu4*, and *Sdu46–Sdu19*). Analysis with Micro-Checker indicated the possibility of null alleles at *Sdu29*.

Twenty-three markers were polymorphic for almaco jacks, and the number of alleles ranged from 2 to 22; H_E ranged from 0.033 to 0.951, while H_O ranged from 0.033 to 0.933. After Bonferroni correction, genotypes at all markers conformed to Hardy–Weinberg expectations and all pairs of microsatellites were in genotypic equilibrium. Analysis with Micro-Checker indicated the possibility of null alleles at *Sdu10*.

Eighteen markers were polymorphic for banded rudderfish. The number of alleles ranged from 2 to 17, H_E ranged from 0.203 to 0.954, and H_O ranged from 0.111 to 1.000. After Bonferroni correction, genotypes at two markers (*Sdu12* and *Sdu31*) deviated significantly from Hardy–Weinberg expectations and one microsatellite pair (*Sdu31–Sdu43*) deviated significantly from genotypic equilibrium. Analysis with Micro-Checker indicated the possibility of null alleles at both *Sdu12* and *Sdu31*.

For fisheries managers, the 30 microsatellites characterized in the present study can be used as tools for assessing stock structure and will provide valuable population genetic indices within each species. The markers will also facilitate the improvement of aquaculture programs through a variety of applications. For aquaculture facilities producing larvae that are subsequently grown for future sale, parentage assignments (Gold et al. 2010) can identify broodstock contribution and can be used to maximize mating design and output. Differences in microsatellite allele sizes between species can be used to authenticate the labeling of aquaculture products as they go to market (Iguchi et al. 2012). As part of a larger set of markers, these microsatellites can be employed for the mapping of quantitative trait loci and the marker-assisted selection of future broodfish (Liu and Cordes 2004).

TABLE 1. Summary data for 30 greater amberjack microsatellite markers that were characterized in lesser amberjacks, yellowtail jacks, almaco jacks, and banded rudderfish. The fluorescently labeled primer is in bold text, with the appropriate label signified by the number of plus signs (+ = 6-FAM; ++ = HEX; +++ = NED). Asterisks indicate information that was taken from earlier descriptions of these microsatellites (Renshaw et al. 2006 for *Sdu1–Sdu27*; Renshaw et al. 2007 for *Sdu29–Sdu46*); all information included for greater amberjacks was published previously (Renshaw et al. 2006, 2007). "N/A" indicates that the marker failed to amplify consistently.

Microsatellite	Primer sequence (5′–3′)[a]*	Repeat sequence*	T_A[b]	Sp[c]	N, N_A[d]	Size range[e]	H_E, H_O[f]	P_{HW}[g]	Micro-Checker[h]
Sdu1	**CGTTTCCATCGCACTTTT**$^{+++}$	$(GACA)_{20}$	53	*Sfa*	19, 4	289–309	0.605, 0.789	0.0363	—
	GCTAACACTCACTGGTG		51	*Sla*	29, 7	352–420	0.808, 0.897	0.0103	—
			49	*Sri*	30, 8	306–362	0.403, 0.333	0.1334	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	28, 11	305–385	0.751, 0.714	0.252	
Sdu2	**CTATATTCACTCTGTTGCC**$^{++}$	$(GAA)_8$	50	*Sfa*	N/A	N/A	N/A	N/A	N/A
	GTGTAGGAGAGACTGTAAG		48	*Sla*	29, 3	135–147	0.327, 0.345	0.4566	—
			46	*Sri*	30, 5	129–144	0.532, 0.433	0.1278	—
				Szo	18, 3	141–150	0.252, 0.278	1.0000	—
				Sdu	29, 5	145–160	0.716, 0.670	0.918	
Sdu3	**CGGTGTATTGTTACTGTGAC**$^{+}$	$(CAA)_8$	53	*Sfa*	19, 2	208–211	0.444, 0.526	0.5997	—
	TCGTCTCTGATTGGTTAG		51	*Sla*	N/A	N/A	N/A	N/A	N/A
			49	*Sri*	30, 4	214–223	0.715, 0.800	0.5003	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	29, 5	212–227	0.602, 0.690	0.717	
Sdu4	**GGAAATAGTTTGGATCACGCTGG**$^{+++}$	$(GACA)_8(GGCA)_4$	60	*Sfa*	N/A	N/A	N/A	N/A	N/A
	GGATGCTCAGTGAAGTTGTGC	$(GACA)_6$	58	*Sla*	29, 3	284–300	0.493, 0.448	0.6056	—
			56	*Sri*	30, 5	281–287[xx]	0.275, 0.267	0.5084	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	29, 10	320–360	0.808, 0.897	0.972	
Sdu5	**GTAAGGATTTGTCATGTAGCC**$^{++}$	$(TAA)_3CAA(TAA)_{10}$	56	*Sfa*	19, 13	250–295	0.899, 0.947	0.8891	—
	GGAGACGAGTTCTCTTTGC	$CAA(TAA)_5$	54	*Sla*	N/A	N/A	N/A	N/A	N/A
			52	*Sri*	30, 1	202	0.000, 0.000	1.0000	—
				Szo	18, 13	203–257	0.921, 0.889	0.3231	—
				Sdu	23, 5	206–236	0.560, 0.478	0.390	
Sdu6	**CCAAAGCAGGTGAAAGTGA**$^{+}$	$(GATA)_{12}$	50	*Sfa*	19, 10	227–267	0.892, 0.842	0.0309	—
	GGTCCATACAACAACTCAG		48	*Sla*	N/A	N/A	N/A	N/A	N/A
			46	*Sri*	30, 11	215–259	0.860, 0.767	0.1031	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	29, 12	231–279	0.844, 0.759	0.043	

(Continued on next page)

TABLE 1. Continued.

Microsatellite	Primer sequence (5′–3′)[a]*	Repeat sequence*	T_A[b]	Sp[c]	N, N_A[d]	Size range[e]	H_E, H_O[f]	P_{HW}[g]	Micro-Checker[h]
Sdu7	**CACTTTCAACTGGAACACC**$^{++}$	$(CAA)_8$	56	*Sfa*	19, 1	337	0.000, 0.000	1.0000	—
	GGTTCTGCTGGCTCATTG		54	*Sla*	N/A	N/A	N/A	N/A	N/A
			52	*Sri*	30, 3	346–352	0.501, 0.367	0.1678	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	26, 3	343–364	0.361, 0.269	0.102	
Sdu8	**CCAGTCTATGAAACACAACC**$^{+}$	$(GAA)_9$	56	*Sfa*	19, 3	101–107	0.152, 0.158	1.0000	—
	CCTGAAGCGATGAAGCGT		54	*Sla*	29, 1	95	0.000, 0.000	1.0000	—
			52	*Sri*	30, 2	101–107	0.033, 0.033	1.0000	—
				Szo	18, 1	95	0.000, 0.000	1.0000	—
				Sdu	29, 3	105–111	0.068, 0.069	1.000	
Sdu9	**CTGTTGTCCTTCCAGAC**$^{+++}$	$(GA)_4(GAA)_9$	53	*Sfa*	19, 8	233–249	0.528, 0.579	0.8750	—
	CCACATCGTCTGAATAGC		51	*Sla*	29, 1	237	0.000, 0.000	1.0000	—
			49	*Sri*	30, 1	219	0.000, 0.000	1.0000	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	29, 2	230–238	0.131, 0.138	1.000	
Sdu10	**CCAAGTCCTCCTGCTACTACCAT**$^{+}$	$(GAA)_{18}$	56	*Sfa*	19, 2	273–276	0.462, 0.474	1.0000	—
	CCTTGTGGATGACCTGTTTG		54	*Sla*	29, 4	261–282	0.577, 0.552	0.8619	—
			52	*Sri*	30, 12	285–321	0.793, 0.633	0.0722	**N**
				Szo	18, 9	287–314	0.887, 0.944	0.9606	—
				Sdu	29, 15	295–346	0.902, 0.966	0.412	
Sdu11	**GCTCTCGTGTGTTACTCAAG**$^{+}$	$(CAA)_7$	56	*Sfa*	19, 1	168	0.000, 0.000	1.0000	—
	GCAACTGTCAGATCCTCCA		54	*Sla*	29, 1	168	0.000, 0.000	1.0000	—
			52	*Sri*	30, 2	168–171	0.066, 0.067	1.0000	—
				Szo	18, 1	168	0.000, 0.000	1.0000	—
				Sdu	29, 2	169–172	0.160, 0.172	1.000	
Sdu12	**CCACAAGTTATCACAAGCCACC**$^{++}$	$(GACA)_5GGCA$	60	*Sfa*	N/A	N/A	N/A	N/A	N/A
	GCTTTGTCCCCTGTGTGCTG	$(GACA)_5$ GGCA	48	*Sla*	N/A	N/A	N/A	N/A	N/A
		$(GACA)_7$	46	*Sri*	30, 7	232–260	0.807, 0.767	0.0759	—
				Szo	18, 9	229–277	0.792, 0.500	***0.0013***	**N**
				Sdu	29, 10	237–313	0.776, 0.690	0.550	

TABLE 1. Continued.

Microsatellite	Primer sequence (5′–3′)[a]*	Repeat sequence*	T_A[b]	Sp[c]	N, N_A[d]	Size range[e]	H_E, H_O[f]	P_{HW}[g]	Micro-Checker[h]
Sdu16	**GAGTTGTACTGTGGTAAAC**$^{+}$	$(CAA)_{11}$	50	*Sfa*	19, 3	108–120	0.198, 0.211	1.0000	—
	GGACATTAGAGTCTGTGG		48	*Sla*	29, 1	102	0.000, 0.000	1.0000	—
			46	*Sri*	30, 2	105–108	0.033, 0.033	1.0000	—
				Szo	18, 3	96–105	0.294, 0.167	0.0397	—
				Sdu	29, 4	114–126	0.462, 0.552	0.174	
Sdu19	**GCATTCTGGCATTAGCAT**$^{+++}$	$(CAGA)_{16}$	56	*Sfa*	19, 7	212–236	0.805, 0.895	0.0441	—
	GGTACTCTAGTTAGCCCTAC		54	*Sla*	29, 2	212–216	0.100, 0.103	1.0000	—
			52	*Sri*	30, 7	232–256	0.848, 0.833	0.0181	—
				Szo	18, 2	212–216	0.203, 0.111	0.1691	—
				Sdu	29, 10	236–272	0.823, 0.724	0.178	
Sdu21	**CTCAGGACAATGTTGGTAG**$^{+}$	$(GATA)_{25}$	56	*Sfa*	19, 16	277–369	0.930, 0.947	0.2709	—
	GCTAACAAGTTCACGACAT		54	*Sla*	29, 10	297–389	0.789, 0.897	0.9997	—
			52	*Sri*	30, 22	289–385[xx]	0.951, 0.867	0.0863	—
				Szo	18, 17	301–389	0.954, 1.000	1.0000	—
				Sdu	27, 20	264–380	0.950, 0.926	0.697	
Sdu22	**CATTCTCCAAGTATGTGACCTC**$^{++}$	$(GAA)_{21}$	56	*Sfa*	19, 6	300–318	0.762, 0.632	0.7447	—
	GCTCTATGCGAATACCTCCA		54	*Sla*	29, 1	292	0.000, 0.000	1.0000	—
			52	*Sri*	30, 1	295	0.000, 0.000	1.0000	—
				Szo	18, 3	295–304	0.586, 0.611	0.2134	—
				Sdu	29, 11	311–341	0.832, 0.862	0.694	
Sdu27	**CCTTCTGTCTTGACTCTGC**$^{+++}$	$(GATA)_{13}$	56	*Sfa*	19, 3	313–317[xx]	0.284, 0.263	0.3553	—
	CGATTCATCCAGCTTTAGG		54	*Sla*	29, 1	303	0.000, 0.000	1.0000	—
			52	*Sri*	30, 7	290–322	0.605, 0.567	0.8688	—
				Szo	18, 8	324–352	0.857, 0.778	0.4006	—
				Sdu	29, 8	266–298	0.783, 0.630	0.060	
Sdu29	**CCTTGCCATACCGATGCCAG**$^{+}$	$(GA)_{14}$	60	*Sfa*	19, 2	299–301	0.235, 0.263	1.0000	—
	GACTGCTCTGCCTGCTTGTTG		58	*Sla*	29, 8	319–365	0.808, 0.345	***0.0000***	**N**
			56	*Sri*	30, 4	311–321	0.571, 0.667	0.4981	—
				Szo	18, 7	309–325	0.765, 0.833	0.3300	—
				Sdu	29, 11	311–377	0.847, 0.793	0.487	

(Continued on next page)

TABLE 1. Continued.

Microsatellite	Primer sequence (5′–3′)[a]*	Repeat sequence*	T_A[b]	Sp[c]	N, N_A[d]	Size range[e]	H_E, H_O[f]	P_{HW}[g]	Micro-Checker[h]
Sdu31	**CACATTTGGACGGATTCTTC**$^{+}$	$(CA)_{14}$	56	*Sfa*	19, 4	84–96	0.745, 0.684	0.4228	—
	GCTGTTATCCTCCAGTGCT		54	*Sla*	29, 9	106–140	0.781, 0.759	0.2719	—
			52	*Sri*	30, 20	90–164	0.941, 0.933	0.2394	—
				Szo	18, 7	86–104	0.578, 0.333	***0.0028***	**N**
				Sdu	29, 7	84–98	0.738, 0.759	0.650	
Sdu32	**CCTGTGAGAGCATTTGGTAT**$^{++}$	$(CA)_{17}$	53	*Sfa*	19, 10	93–125	0.851, 0.789	0.5653	—
	GTGCTTGTCTCTTCTGTCAT		51	*Sla*	29, 9	97–131	0.653, 0.862	0.8653	—
			49	*Sri*	30, 8	99–165	0.811, 0.700	0.1963	—
				Szo	18, 12	103–133	0.921, 0.944	0.8969	—
				Sdu	29, 21	99–177	0.948, 0.862	0.231	
Sdu33	**CCTCTAACAGCCACAATCA**$^{++}$	$(GA)_{13}$	56	*Sfa*	19, 7	194–212	0.802, 0.789	0.8488	—
	GCTCTTCACCTTCCTCATA		54	*Sla*	29, 2	210–212	0.290, 0.345	0.5616	—
			52	*Sri*	30, 5	194–202	0.671, 0.600	0.4063	—
				Szo	18, 7	188–216	0.838, 0.833	0.4069	—
				Sdu	29, 3	202–208	0.296, 0.276	0.598	
Sdu34	**CCTTGTGTTGTATCTGCTGTAA**$^{+++}$	$(GA)_{20}$	56	*Sfa*	19, 4	93–103	0.681, 0.737	0.8497	—
	GGAATAAACCTCGTCTGTCA		54	*Sla*	29, 1	194	0.000, 0.000	1.0000	—
			52	*Sri*	30, 3	91–97	0.532, 0.567	0.5847	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	29, 8	84–118	0.778, 0.517	0.028	
Sdu36	**CTGTTATGAAGCAGTGAAGAGG**$^{+}$	$(GA)_{23}$	56	*Sfa*	19, 3	194–198	0.522, 0.474	0.4838	—
	GGACCATCCTGCTCTGACA		54	*Sla*	29, 1	186	0.000, 0.000	1.0000	—
			52	*Sri*	30, 16	192–250	0.912, 0.933	0.4456	—
				Szo	18, 4	198–210	0.487, 0.500	0.3928	—
				Sdu	29, 9	200–226	0.815, 0.690	0.132	
Sdu37	**CCTCTAATGGACTTCAGCG**$^{+++}$	$(CA)_{16}$	53	*Sfa*	19, 8	164–180	0.858, 0.842	0.3169	—
	GGTTATTTTGAGAGCCGTC		51	*Sla*	29, 14	173–281	0.859, 1.000	0.2088	—
			49	*Sri*	N/A	N/A	N/A	N/A	N/A
				Szo	18, 12	167–219	0.887, 0.833	0.5094	—
				Sdu	29, 25	160–278	0.889, 0.828	0.448	

TABLE 1. Continued.

Microsatellite	Primer sequence (5′–3′)[a]*	Repeat sequence*	T_A[b]	Sp[c]	N, N_A[d]	Size range[e]	H_E, H_O[f]	P_{HW}[g]	Micro-Checker[h]
Sdu39	**AGTGGCTTCTGCTGCTGT**$^{++}$	$(CA)_{16}$	56	*Sfa*	19, 4	145–153	0.642, 0.684	1.0000	—
	CGTGTGCGTGCTTGTAAA		54	*Sla*	29, 2	137–139	0.242, 0.276	1.0000	—
			52	*Sri*	30, 4	137–147	0.653, 0.533	0.0491	—
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	29, 6	154–180	0.338, 0.345	0.688	
Sdu40	**CGATGCTTTCAACTCCGACACAC**$^{+++}$	$(CA)_{17}$7bp $(CA)_5$	64	*Sfa*	19, 7	187–201	0.805, 0.737	0.1863	—
	CCATCCTTCATCAGCAACAACATCC		62	*Sla*	29, 3	181–185	0.617, 0.655	0.7009	—
			60	*Sri*	30, 12	196–232	0.838, 0.900	0.3556	—
				Szo	18, 3	189–193	0.541, 0.778	0.0975	—
				Sdu	28, 9	197–235	0.825, 0.893	0.884	
Sdu41	**AGCGTGGACAGTTTATGG**$^{++}$	$(CA)_{18}$	53	*Sfa*	19, 6	96–112	0.718, 0.842	0.3794	—
	GTCTGTTTACTGGTCGCA		51	*Sla*	N/A	N/A	N/A	N/A	N/A
			49	*Sri*	N/A	N/A	N/A	N/A	N/A
				Szo	N/A	N/A	N/A	N/A	N/A
				Sdu	29, 10	96–130	0.636, 0.724	0.548	
Sdu43	GGAACATTTGGAGCCATAAGAC	$(CA)_{28}$	60	*Sfa*	19, 5	259–271	0.518, 0.579	0.8453	—
	CAGAAGAAGAGCGTGGTGGAGAG^{+++}		58	*Sla*	29, 10	283–315	0.845, 0.931	0.2253	—
			56	*Sri*	30, 22	272–340	0.941, 0.900	0.6472	—
				Szo	18, 14	265–305	0.913, 0.833	0.1897	—
				Sdu	29, 11	276–298	0.835, 0.828	0.654	
Sdu44	**GGTAATGGGAGGTGTGAGTGT**$^{++}$	$(GA)_{12}$	56	*Sfa*	19, 3	118–124	0.656, 0.579	0.5681	—
	CCTTCTCCTGTTAATCCATCTCC		54	*Sla*	29, 2	117–119	0.100, 0.103	1.0000	—
			52	*Sri*	30, 1	114	0.000, 0.000	1.0000	—
				Szo	18, 10	116–136	0.813, 0.722	0.6769	—
				Sdu	29, 3	114–124	0.402, 0.379	0.574	
Sdu46	**GCAGTGTGAGCCATACATTAC**$^{+++}$	$(GA)_{30}$	53	*Sfa*	19, 2	238–252	0.053, 0.053	1.0000	—
	CTACAGGACAAAAGCCATT		51	*Sla*	29, 5	232–244	0.749, 1.000	***0.0003***	**—**
			49	*Sri*	N/A	N/A	N/A	N/A	N/A
				Szo	18, 1	211	0.000, 0.000	1.0000	—
				Sdu	29, 13	217–259	0.817, 0.897	0.628	

[a]Primer sequences are forward (top) and reverse (bottom).

[b]T_A is the annealing temperature (°C) used for PCR amplification (see Methods text).

[c]Species (Sp) characterized are the lesser amberjack (*Sfa*), yellowtail jack (*Sla*), almaco jack (*Sri*), banded rudderfish (*Szo*), and greater amberjack (*Sdu*).

[d]N is the number of individuals assayed; N_A is the number of alleles detected.

[e]Size range (bp) refers to alleles that have been discovered thus far; the superscript "xx" indicates that alleles were not spaced as anticipated (*Sdu4*, *Sdu21*, and *Sdu27*).

[f]H_E is expected heterozygosity; H_O is observed heterozygosity.

[g]P_{HW} is the probability of deviation from Hardy–Weinberg expectations; deviations that were significant after Bonferroni correction (Rice 1989) are shown in bold italics.

[h]Possible issues with loci as indicated by Micro-Checker software (Van Oosterhout et al. 2004): **N** = evidence for null alleles; "–" = no evident issues. Micro-Checker was not utilized with the previously published data for greater amberjacks.

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