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Research Article

Parental contribution in a cultivated stock for the spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869) estimated by newly developed microsatellite markers

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ABSTRACT. The spotted rose snapper *Lutjanus guttatus* is a fishery relevant species from the eastern Pacific Ocean, with aquaculture potential. Species-specific genetic markers are needed for the genetic characterization of wild and cultivated populations to help management strategies. Eighteen hypervariable microsatellites were developed by Next Generation Sequencing and characterized in a wild population sample. Genetic diversity was high (observed heterozygosity = 0.88 ± 0.050; the number of alleles per locus = 13.4 ± 1.3) and few loci departed from the Hardy-Weinberg Equilibrium, leaving 14 loci potentially suitable for population genetic studies. A reduced panel of five loci was tested in a cultivated stock to determine the parentage of progeny (embryonated eggs; n = 413), to estimate the temporal contribution of each parental broodstock. The above resulted in the successful assignment of 95.6% of the progeny to its parental couple, representing 17 out of the 24 possible families. Two of the four females produced most of those progeny (97.3%). These females, which reproduced throughout the season, did not spawn on consecutive days. The contribution of males was evenly distributed during the season and occurred on successive days. Some microsatellites can be used in other lutjanids (*L. peru*, *L. argentiventris*, and *Hoplopagrus guentherii*).

Keywords: *Lutjanus guttatus*; population genetics; embryonic eggs; genetic markers; parentage assessment; reproductive performance

INTRODUCTION

The spotted rose snapper, *Lutjanus guttatus* (Steindachner, 1869), is a demersal marine finfish with a wide distribution range along the eastern Pacific Ocean from the Gulf of California in Mexico to Ecuador (Fischer *et al*., 1995). It is a valuable fishing resource in the region (Herrera-Ulloa *et al*., 2010; Sarabia-Méndez *et al*., 2010; Correa-Herrera & Jiménez-Segura, 2013), with a high potential for aquaculture (Ibarra-Castro *et al*., 2013). *Lutjanus guttatus* is a batch spawner with asynchronous ovarian development during a long reproductive season comprising peak spawning periods in April, August and October (Arellano-Martínez *et al*., 2001; Sarabia-Méndez *et al*., 2010). Little is known regarding the spawning contribution of females and males daily.

Microsatellite genetic markers are a necessary tool for the genetic characterization of wild populations that can be used, for example, to improve their management, in rehabilitation programs, and stock identification (Hallerman, 2003). They are also important in aquaculture as they can be used to determine female spawning frequency. Thus, the effective parental contribution [*e.g*., red sea bream *Pagrus major* (Perez-Enriquez *et al*., 1999), California yellowtail *Seriola lalandi* (Smith *et al*., 2015), gilthead seabream *Sparus aurata* (García-Fernández *et al*., 2018)], which due

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the disproportion in male to female contributions, large family size variance and null female spawners in the broodstock can lead to the potential accumulation of inbreeding within the hatchery (Blonk *et al*., 2009; Domingos *et al*., 2014). Other markers, such as Single Nucleotide Polymorphisms (SNPs), have shown reliable results for parentage testing in aquaculture species [*e.g.*, shrimp (Perez-Enriquez & Max-Aguilar, 2016)]. However, for *L. guttatus* there is no previous genomic information available.

The present study aimed to obtain a set of microsatellite markers for future population genetic studies of *L. guttatus* and to test a reduced panel to estimate the temporal parental contribution in a cultivated stock of the species.

MATERIALS AND METHODS

Biological material

Fin clips of 10 *Lutjanus guttatus* individuals were collected in 2011 at the eastern coast of the Baja California Peninsula, Mexico, and preserved in 70% ethanol. Genomic DNA was obtained (Aljanabi & Martinez, 1997), and a DNA mix was sent to the Savannah River Ecology Laboratory, University of Georgia, U.S.A., for microsatellite screening by Next Generation Sequencing (Illumina library preparation and sequencing, bioinformatics analysis and primer design). A set of 48 primer pairs (tetra- and pentanecleotides) was tested in these 10 individuals. PCR was done in volumes of 11 µL containing 1 µL DNA as template (20 ng μL^{-1}), 1 × *Taq* Buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.4 µM of each forward and reverse primers (Macrogen, Korea), 0.025 U μL^{-1} *Taq* polymerase (Promega, UK), and Milli-Q water. PCR thermal conditions (C1000 thermal cycler, Bio-Rad) were: 94ºC for 2 min; 30 cycles at 94ºC for 45 s, annealing temperature for 45 s, and 72ºC for 1 min; then a final extension at 72ºC for 10 min. The annealing temperature for each primer was calculated using the formula $T_a = 4(C+G) + 2(A+T) - 5$. The PCR products were separated on polyacrylamide gel electrophoresis (5%, 7.5 M urea; 1800 V, 50 mA, and 50 W). Fragments were visualized using Sybr-Gold within a 1% agarose matrix and scanned (FMBIOIII, Hitachi).

Genetic markers selection

A set of 18 microsatellite loci, showing reliable amplification patterns, was selected for characterization on the same 10 individuals, and their sequences (Macrogen) were deposited in GenBank (Table 1). PCR reactions were done in 20 µL volumes with the use of an M13 primer (5'-TGTAAAACGACGGCCAGT) labeled with the fluorophores 6-FAM, VIC, NED or PET at 1.6 µM, reverse primers at 1.6 µM, and forward primers having an extension of the M13 sequence at the 5'-end at 0.4 µM (Schuelke, 2000) (Table 1). The rest of the components were at the same concentrations as above. The amplification conditions were the same as above, but the final extension was set with eight additional cycles of 94ºC for 30 s, M13-annealing at 53ºC for 45 s, and 72ºC for 45 s. Two µL of PCR products were added with 0.25 µL of LIZ500 Size Standard (Applied Biosystems) and 9.75 µL de HiDi-formamide, placed in a 96-well microplate and put into the ABI 3130 automated DNA sequencer. The genotypes were obtained using the software Gene Mapper version 4.0 (Applied Biosystems).

Allele frequencies per locus were calculated with the program Arlequin version 3.5 (Excoffier & Lischer, 2010), and used to estimate genetic diversity parameters [number of alleles per locus; observed (Ho) and expected (He) heterozygosities] and Hardy-Weinberg Equilibrium (HWE) (Exact test using a Markov chain: 50,000 dememorizations, 100,000 steps). The potential presence of null alleles, stuttering, or allele drop-out was assessed with the program Micro-Checker (Van-Oosterhout *et al*., 2004).

Cross-amplification of microsatellites was tested in three lutjanid species: red snapper *Lutjanus peru* (Nichols & Murphy, 1922) (n = 5), yellow snapper *Lutjanus argentiventris* (Peters, 1869) (n = 4), and greenbar snapper *Hoplopagrus guentherii* (Gill, 1862) (n = 1), all collected from the Gulf of California.

Broodstock management

The 10 individuals of *L. guttatus* described in the previous section (six males, four females) were kept in a maturation tank equipped with an external spawn collector, at the Centro Interdisciplinario de Ciencias Marinas-IPN, México. They were fed daily at satiation with sardines and squid. During the reproductive season of 2011 (June-October), spontaneous spawning was obtained. For each collected spawn, viable embryonated eggs were separated from dead eggs by buoyancy. A fraction of those was collected and preserved in 1.5 mL microcentrifugation tubes with 70% ethanol. From a total of 36 spawns, the embryonated eggs from 14 spawning events were sampled for DNA analysis (12, 13, 14 July; 4, 14, 22 August; 8, 9, 10 September; 14, 15, 16, 20, 21 October).

The embryonated eggs were individually separated using a microscope (Olympus CX31), and only those from the late gastrula developmental stage were selected, as earlier stages failed to amplify PCR products adequately. Remnants of ethanol were evapo-

Table 1. Characterization of a set of 18 microsatellites isolated from *Lutjanus guttatus* (F: forward; R: reverse). M13 is the sequence 5'TGTAAAACGACGGCCAGT included at the beginning of the forward primer. Fl: fluorophore; Ta: annealing temperature; n_a: number of alleles per locus; Ho: observed heterozygosity; He: expected heterozygosity; HWE: the probability of deviation from Hardy-Weinberg Equilibrium; Bonf: Significance after Bonferroni correction (N.S.: non-significant; *significant values); N.A.: not available. CA: positive cross-amplification with *Lutjanus peru* (Lp), *Lutjanus argentiventris* (La) and *Hoplopagrus guentherii* (Hg). GenBank accession numbers are provided.

Locus	Primer sequence (5'-3')	Repeat motif	Fl	Ta (°C)	Allelic size range (pb)	n_a	Ho	He	HWE	Bonf	GenBank accesion	CA
Lgut07	F: M13-TGATAATAACCATGCCCATATTTCC	ATCT	PET	63	270-338	10.0	0.778	0.902	0.141	N.S.	MF416120	Lp
	R: GCTTGTTCAGATTCAACCGC											
Lgut15 [b]	F: M13-ACTCTGGTCTGGAGATTGGG	ATGG	PET	58	259-327	14.0	0.900	0.963	0.443	N.S.	MF416121	Lp, La
	R: TCAATCACGACAACAGTGACG											
Lgut16 [b]	F: M13-GAGGTGTCTGTAATCTACAAATTCACC	ATCT	PET	63	233-331	13.0	1.000	0.958	1.000	N.S.	MF416122	--
	R: TCAACATTCTAACTGACTGTTTAGGC											
Lgut18 [a,b]	F: M13-AAACACTGGGTCTGGGTTGG	AAAG	6FAM	56	235-317	14.0	0.900	0.947	0.620	N.S.	MF416123	Lp, La
	R: TCAACACTTGTTGGCTTCCC											
Lgut19 [b]	F: M13-TGAATCAGCGACTCTGACAGC	ATCT	NED	58	291-422	18.0	0.900	0.984	0.185	N.S.	MF416124	Lp, La, Hg
	R: ACCCAGACTGGCTGTGCC											
Lgut21 [a,b]	F: M13-AAGGAGGACTTTATTCCATCAGC	ATCT	6FAM	60	231-363	14.0	0.900	0.947	0.561	N.S.	MF416125	Lp, La
	R: GGTGGACAGTTGGTTCATCC											
Lgut26 [b]	F: M13-CCATCCTGGTTAGGTTGTTGC	ATCT	VIC	63	242-305	14.0	0.800	0.963	0.055	N.S.	MF416126	Lp, La, Hg
	R: GCAACCAAGAACTTCACTGTAACC											
Lgut30 [a,b]	F: M13-TCCTTTACATAGTTGTAATTGAGGAGG	ATCT	VIC	63	436-502	12.0	0.900	0.947	0.545	N.S.	MF416127	--
	R: ATCGGCACTATTGCATGTGG											
Lgut32	F: M13-TCTTGCACACCAGATTCTTATGG	ATCT	NED	63	181-311	17.0	0.700	0.984	< 0.001	*	MF416128	--
	R: TCAAGATTTAAAGAGACATTTCACCC											
Lgut34 [a,b]	F: M13-GGTTTATTCAAATACACTGGTGCC	ATCT	PET	60	371-430	13.0	1.000	0.947	1.000	N.S.	MF416129	Lp, La
	R: CAGCTCCAAACCACTTCGC											
Lgut37 [b]	F: M13-TTTCAAGGGCATTTATGTGGC	ATCT	VIC	58	364-424	10.0	0.800	0.911	0.150	N.S.	MF416130	Lp
	R: AAGATGCTCCGTAAGGTATCGC											
Lgut38 [b]	F: M13-AAGAACTCTTGAGACAGTTGGGC	ATCT	PET	60	207-303	13.0	0.900	0.947	0.605	N.S.	MF416131	Lp
	R: TGTGTTTGTGTGAATTCTTGGC											
Lgut39 [a,b]	F: M13-AGGTCACATGACGACAGACG	ATCT	6FAM	56	264-326	12.0	1.000	0.932	1.000	N.S.	MF416132	Hg
	R: TGCAGCTTTAAACATCCACG											
Lgut40	F: M13-AAGACTATCGACTGCTGGTGTCC	ATGG	VIC	63	226-335	13.0	0.700	0.953	0.005	N.S.	MF416133	--
	R: GCAAAGTTAGGGCACAACATCC											
Lgut43 [b]	F: M13-TTTGGGAATTATGTTCATTTGC	ATCT	6FAM	56	236-292	11.0	0.900	0.942	0.638	N.S.	N.A. [c]	Lp
	R: ATGCAAATGTTGTGCCTGC											
Lgut44 [b]	F: M13-CGTGTCACATCTCTGTGTTAATGC	AAGAG	NED	63	263-352	17.0	0.800	0.984	0.026	N.S.	MF416134	Lp, La
	R: TGACGCGTCTCTGATTACCC											
Lgut46 [b]	F: M13-AAGGACAGCAAAGAGGCTCG	ACGTG	NED	58	215-270	10.0	1.000	0.916	0.822	N.S.	MF416135	--
	R: GAGCTGCACATCAGGAGGG											
Lgut47	F: M13-GCTGTCATCAACGCTACTGC	ATATT	6FAM	56	236-297	16.0	0.900	0.968	0.445	N.S.	MF416136	--
	R: AAATGGGCCTCAAATGGC											
	Mean	--		--	--	13.4	0.877	0.950	--	--		

[a] Loci used for the parental contribution analysis

[b] Loci suitable for population genetics studies

[c] The amplification of this locus for sequencing failed

rated, and embryonated eggs were put into individual tubes with 18 µL of MilliQ water. They were preserved at -20ºC. For DNA release, embryonated eggs were unfrozen, smashed with a plastic pestle and centrifuged at 1,533 g for 1 min. The supernatant was used as a DNA template. A total of 32 embryonated eggs from each of the 14 spawning events were used for genotyping.

Parentage testing

Based on their polymorphism, allelic range, electropherogram peak quality and the possibility of multiplexing, five loci were selected for genotyping (Table 1). The forward primer (without the M13 extension) from the microsatellite was labeled with a fluorescent label at 5' (Thermo Fisher Scientific) (6FAM-*Lgut18*, PET-*Lgut21*, 6FAM-*Lgut30*, NED-*Lgut34* and VIC-*Lgut39*). For adults, PCR multiplex reactions were conducted in 21 µL volumes containing 1 µL DNA (20 ng μL^{-1}), 1 × *Taq* buffer, 1.5 mM $MgCl_2$, 0.35 mM dNTPs, 0.3 µM of each primer and 0.07 U μL^{-1} *Taq* polymerase. For embryonated eggs, PCR reactions were done using the same quantities but in a volume of 23 µL with 3 µL of DNA. PCR thermal conditions were as follows: 94ºC for 2 min, 42 cycles of 94ºC for 45 s, 60ºC for 45 s and 72ºC for 1 min, and a final extension at 72ºC for 10 min. Products were electrophoresed on an ABI 3130 automated DNA sequencer. Alleles were sized using the LIZ500 Size Standard (Applied Biosystems) and read using GeneMapper 4.0 software (Applied Biosystems).

The combined non-exclusion probability for the five loci set was estimated by the program Cervus 3.0.7 (Kalinowski *et al*., 2007). Parentage analyses for each of the 14 spawning events were performed by probabilistic and direct exclusion approaches using Cervus 3.0.7 (Kalinowski *et al*., 2007) and Vitassign (Vandeputte *et al*., 2006), respectively, to estimate the number of contributing males and females. Those cases, in which the parentage assignment by Cervus and Vitassign coincided. Still, there were some loci showing mismatches; they were treated as putative mutations either by the change in the number of repeats or by null alleles. The mutation rate per locus was calculated, dividing the number of mutations by twice the number of genotypes in the progeny at each locus. The mutation rate was also calculated for males and females.

RESULTS

The 18 microsatellite loci showed reliable genotyping patterns in the *Lutjanus guttatus* broodstock, resulting in high genetic diversity (n_a = 13.4 ± 1.3; *H*o = 0.88 ± 0.05; Table 1). Three loci departed from HWE (only one after the Bonferroni correction) (Table 1), which can be explained by the potential presence of null alleles, rather than by stuttering or allele drop-out, as indicated by the Micro-Checker analysis. Fourteen loci are available to assess population genetic structure in wild *L. guttatus* (N. Diaz-Viloria, *unpublish. data*), and several loci are potentially useful for the other snapper species (Table 1).

For parentage assignment, 413 embryonated eggs were used. The combination of direct and probabilistic (95% CL) exclusion methods resulted in 95.6% of the progeny (n = 395) assigned to a single parental couple, leaving 4.3% unassigned. Seventeen families (out of 24) were represented in the progeny (Table 2).

The reproductive season within the breeding tank spanned from June to November, with a peak number of spawns occurring in October. Most males (7A62-M, 1170-M, 1538-M and 2924-M) reproduced throughout the season and during consecutive days (Fig. 1a). In contrast, most of the progeny (n = 384; 97.2%) were produced by only two of the four females (4B67-H and 4953-H), and spawning did not occur on consecutive days (Fig. 1b), indicating that females (at least 4B67-H) spawn every other day.

Unexpected genotypes were observed in several families, resulting in a deviation from the expected Mendelian proportions (Table 3). As it is unlikely that these genotypes come from genotyping errors (the sequencer sizing differences observed in four duplicated samples was between 0-0.7 units in at least four loci), they appear to be a consequence of both null and mutated alleles. Considering a null allele as a mutation event, the mutation rate per locus varied between a maximum of 1.2×10^{-1} in *Lgut21* to a minimum of 7.7×10^{-3} in *Lgut34*, for a mean of 4.5×10^{-2}. Null-allele events were three times larger than changes in the number of repeats. While all the mutations in *Lgut21* and *Lgut39* were due to null alleles, there was a combination of null alleles and base pairs gains in the other loci (Table 3). In *Lgut18* and *Lgut30*, the most common change was a gain in four base pairs (equivalent to one microsatellite repeat). The mutation rates were, on average, almost twice higher for females than males (Table 4).

DISCUSSION

The usefulness of microsatellites as genetic markers for parentage assignment has been demonstrated in more than 20 cultivated fish species (Yue & Xia, 2014). The capability of correct assignment is dependent on several characteristics of the genetic markers, of which their

Table 2. The number of progenies assigned to each potential family in the spawning events of July to October, using the probabilistic (95% confidence level) and direct exclusion methods.

Female	Male	Number of individuals	Female	Male	Number of individuals
4953_H	1170_M	54	4B67_H	1170_M	61
	1538_M	8		1538_M	46
	2924_M	39		2924_M	30
	6720_M	14		6720_M	8
	7A62_M	54		7A62_M	53
	3C42_M	2		3C42_M	15
2C30_H	1170_M	0	IF70_H	1170_M	0
	1538_M	1		1538_M	0
	2924_M	4		2924_M	1
	6720_M	0		6720_M	0
	7A62_M	3		7A62_M	0
	3C42_M	2		3C42_M	0
Total number of individuals					395
Total number of families with progeny					17

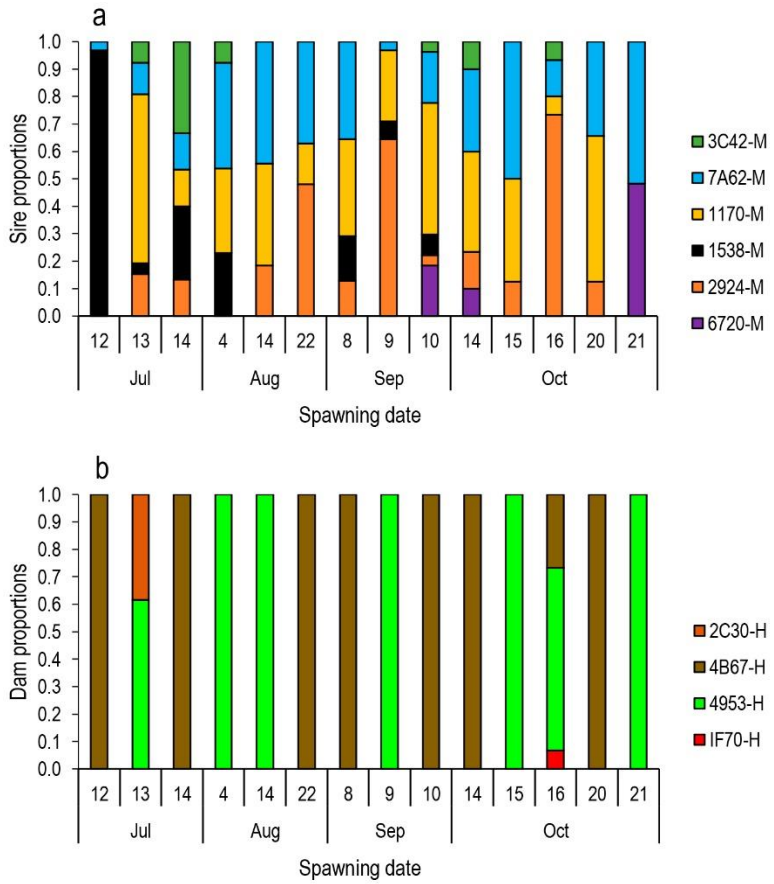

Figure 1. The proportion of breeders contributing to progeny during the spawning events of July-October 2011. a) Males, b) females.

variability is one of the most relevant (Vandeputte & Haffray, 2014). The five high-variable microsatellites selected (with a combined probability of non-exclusion in the order of 10^{-6}) were enough to confidently determine, by both exclusion methods, the parentage of 92% of the progeny from a relatively small *Lutjanus*

Table 3. Goodness of fit test ($\chi 2$) for Mendelian inheritance proportions of genetically assigned progeny in *Lutjanus guttatus* broodstock. n: number of progeny in the family. Families with four or less progeny not shown. UP: unexpected genotypes. MA: probably mutated allele with its ancestor (S: male, D: female) and base pair difference in parenthesis. [a]Unexpected genotypes not considered; *Significant values ($P < 0.05$).

Family	Locus	Parental genotypes		n	Genotypes in the progeny and observed number of individuals in parenthesis				$\chi 2$	Unexpected genotypes	n	Mutated or null allele
		Male	Female									
3C42-M / 4B67-H	*Lgut18*	247/263	247/251	14	247/247 (2)	247/251 (1)	263/247 (7)	263/251 (4)	6.0[a]	251/267	1	263 (S, 4)
	Lgut21	275/349	248/307	12	275/248 (0)	275/307 (5)	349/248 (1)	349/307 (6)	8.7[a]	349/349	2	248 (D, null)
	Lgut30	427/475	448/464	13	427/448 (2)	427/464 (6)	475/448 (2)	475/464 (3)	3.3[a]	431/464	1	427 (S, 4)
	Lgut34	383/389	376/385	15	383/376 (9)	383/385 (2)	389/376 (2)	389/385 (2)	9.8*	--		--
	Lgut39	272/281	248/264	15	272/248 (7)	272/264 (1)	281/248 (3)	281/264 (4)	5.0	--		--
7A62-M / 4B67-H	*Lgut18*	218/238	247/251	53	218/247 (11)	218/251 (14)	238/247 (15)	238/251 (13)	0.7	--		--
	Lgut21	250/287	248/307	33	250/248 (2)	250/307 (15)	287/248 (3)	287/307 (13)	16.3*,[a]	250/250	9	248 (D, null)
										287/287	11	
	Lgut30	444/472	448/464	44	444/448 (8)	444/464 (11)	472/448 (16)	472/464 (9)	3.5[a]	448/464	3	444 (S, 4)
										448/475	1	472 (S, 3)
										456/472	1	448,464 (D, 8, -8)
										464/464	1	444,472 (S, null)
	Lgut34	381/411	376/385	52	381/376 (11)	381/385 (17)	411/376 (8)	411/385 (16)	4.2	376/385 (1)		381 (S, -5 or 4)
	Lgut39	264/297	248/264	53	264/248 (8)	264/264 (14)	297/248 (17)	297/264 (14)	3.2	--		--
7A62-M / 4953-H	*Lgut18*	218/238	226/284	54	218/226 (13)	218/284 (16)	238/226 (13)	238/284 (12)	0.7	--		--
	Lgut21	250/287	270/291	53	250/270 (16)	250/291 (9)	287/270 (16)	287/291 (12)	2.6	291/291	1	250,287 (S, null)
	Lgut30	444/472	448/452	49	444/448 (10)	444/452 (11)	472/448 (12)	472/452 (16)	1.7	448/452	1	444 (S, 4)
	Lgut34	381/411	378/396	54	381/378 (19)	381/396 (7)	411/378 (13)	411/396 (15)	5.6	--		--
	Lgut39	264/297	285/289	54	264/285 (14)	264/289 (16)	297/285 (13)	297/289 (11)	0.96	--		--
1170-M / 4B67-H	*Lgut18*	255/296	247/251	61	255/247 (17)	255/251 (15)	296/247 (15)	296/251 (14)	0.31	--		--
	Lgut21	217/287	248/307	35	217/248 (2)	217/307 (14)	287/248 (4)	287/307 (15)	15.4*,[a]	217/217	10	248 (D, null)
										287/287	16	
	Lgut30	420/440	448/464	53	420/448 (17)	420/464 (8)	440/448 (15)	440/464 (13)	3.4[a]	431/464	1	420 (S, 11) or 440 (S, -9)
										440/440	2	448,464 (D, null)
										440/452	1	448 (D, 4)
										440/456	1	448,464 (D, 8, -8)
	Lgut34	391/411	376/385	60	391/376 (16)	391/385 (17)	411/376 (12)	411/385 (15)	0.4[a]	376/385	1	391 (S, -15, -6) or 411 (S, -35, -26)
	Lgut39	264/272	248/264	61	264/248 (19)	264/264 (13)	272/248 (12)	272/264 (17)	2.14	--		--
1170-M / 4953-H	*Lgut18*	255/296	226/284	49	255/226 (17)	255/284 (8)	296/226 (10)	296/284 (14)	4.0	230/255	4	226 (D, 4)
										230/296	1	
	Lgut21	217/287	270/291	51	217/270 (15)	217/291 (13)	287/270 (10)	287/291 (13)	1.0	270/270	1	217, 287 (S, null)
	Lgut30	420/440	448/452	33	420/448 (7)	420/452 (9)	440/448 (9)	440/452 (8)	0.3	420/456	2	452 (D, 4)
										444/448	1	440 (S, 4)
	Lgut34	391/411	378/396	51	391/378 (11)	391/396 (13)	411/378 (15)	411/396 (12)	0.7	411/411	1	378, 396 (D, null)
	Lgut39	264/272	285/289	51	264/285 (13)	264/289 (13)	272/285 (8)	272/289 (17)	3.2	264/264	3	285, 289 (D, null)

continuation

Family	Locus	Parental genotypes		n	Genotypes in the progeny and observed number of individuals in parenthesis				χ^2	Unexpected genotypes	n	Mutated or null allele
1538-M / 4B67-H	*Lgut18*	230/251	247/251	46	230/247 (12)	230/251 (17)	251/247 (17)	251/251 (0)	16.8*	--		--
	Lgut21	286/307	248/307	35	286/248 (0)	286/307 (11)	307/248 (0)	307/307 (24)	44.7*,a	286/286(11)		248 (D, null)
	Lgut30	464/479	448/464	36	464/448 (9)	464/464 (14)	479/448 (5)	479/464 (8)	4.7 *,a	448/483 (2); 452/464 (1); 452/479 (1); 456/479 (1); 464/483 (1); 464/486 (1); 467/479 (1)		479 (S, 4); 448 (D, 4); 448 (D, 4); 448 (S, 8) or 464 (S, -8); 479 (S, 4); 479 (S, 7); 464 (D, 3)
	Lgut34	363/378	376/385	46	363/376 (13)	363/385 (12)	378/376 (9)	378/385 (12)	0.78	--		--
	Lgut39	268/272	248/264	46	268/248 (8)	268/264 (14)	272/248 (14)	272/264 (10)	2.3	--		--
1538-M / 4953-H	*Lgut18*	230/251	226/284	8	230/226 (3)	230/284 (0)	251/226 (2)	251/284 (3)	3.0	--		--
	Lgut21	286/307	270/291	8	286/270 (3)	286/291 (2)	307/270 (0)	307/291 (3)	3.0	--		--
	Lgut30	464/479	448/452	6	464/448 (3)	464/452 (1)	479/448 (1)	479/452 (1)	2.0	--		--
	Lgut34	363/378	378/396	8	363/378 (1)	363/396 (2)	378/378 (3)	378/396 (2)	1.0	--		--
	Lgut39	268/272	285/289	8	268/285 (1)	268/289 (0)	272/285 (6)	272/289 (1)	11.0	--		--
2924-M / 4B67-H	*Lgut18*	267/271	247/251	18	267/247 (0)	267/251 (0)	271/247 (13)	271/251 (5)	25.1*,a	247/247 (5); 251/251 (7)		267 (S, null)
	Lgut21	262/300	248/307	19	262/248 (2)	262/307 (11)	300/248 (0)	300/307 (6)	14.9*,a	262/262 (3); 300/300 (6)		248 (D, null)
	Lgut30	431/483	448/464	30	431/448 (7)	431/464 (9)	483/448 (6)	483/464 (8)	0.67	--		--
	Lgut34	376/391	376/385	30	376/376 (7)	376/385 (5)	391/376 (11)	391/385 (7)	2.5	--		--
	Lgut39	277/293	248/264	30	277/248 (5)	277/264 (5)	293/248 (13)	293/264 (7)	5.7	--		--
2924-M / 4953-H	*Lgut18*	267/271	226/284	18	267/226 (0)	267/284 (0)	271/226 (11)	271/284 (7)	19.8*,a	226/226(13); 230/271 (1); 284/284 (7)		267 (S, null); 226 (D, 4); 267 (S, null)
	Lgut21	262/300	270/291	39	262/270 (11)	262/291 (9)	300/270 (9)	300/291 (10)	0.28	--		--
	Lgut30	431/483	448/452	39	431/448 (7)	431/452 (15)	483/448 (8)	483/452 (9)	4.0	--		--
	Lgut34	376/391	378/396	39	376/378 (7)	376/396 (12)	391/378 (7)	391/396 (13)	3.2	--		--
	Lgut39	277/293	285/289	39	277/285 (11)	277/289 (9)	293/285 (10)	293/289 (9)	0.28	--		--
6720-M / 4B67-H	*Lgut18*	251/300	247/251	8	251/247 (5)	251/251 (1)	300/247 (0)	300/251 (2)	7.0	--		--
	Lgut21	270/283	248/307	6	270/248 (1)	270/307 (3)	283/248 (2)	283/307 (0)	3.3*, a	283/283 (2)		248,307 (D, null)
	Lgut30	431/440	448/464	8	431/448 (0)	431/464 (3)	440/448 (4)	440/464 (1)	5.0	--		--
	Lgut34	354/380	376/385	8	354/376 (2)	354/385 (3)	380/376 (2)	380/385 (1)	1.0	--		--
	Lgut39	281/301	248/264	8	281/248 (2)	281/264 (3)	301/248 (2)	301/264 (1)	1.0	--		--
6720-M / 4953-H	*Lgut18*	251/300	226/284	14	251/226 (2)	251/284 (3)	300/226 (6)	300/284 (3)	2.6	--		--
	Lgut21	270/283	270/291	14	270/270 (4)	270/291 (5)	283/270 (3)	283/291 (2)	1.4	--		--
	Lgut30	431/440	448/452	13	431/448 (4)	431/452 (3)	440/448 (2)	440/452 (4)	0.85[a]	444/448 (1)		440 (S, 4)
	Lgut34	354/380	378/396	12	354/378 (2)	354/396 (0)	380/378 (8)	380/396 (2)	12.0*,a	356/396 (2)		354 (S, 2)
	Lgut39	281/301	285/289	14	281/285 (5)	281/289 (2)	301/285 (2)	301/289 (5)	2.6	--		--

Table 4. Number of genotypes per parent (progeny per parent × 5 loci), non-scored genotypes from progeny, number of mutated and null alleles, and estimated mutation rate.

Parent	ID	Genotypes per parent	Non-scored progeny genotypes	Mutations	Null	Mutation rate ($\times 10^{-2}$)
Male	3C42-M	95	3	2	0	2.2
	7A62-M	550	7	6	2	1.5
	1170-M	590	31	3	2	0.9
	1538-M	275	2	5	0	1.8
	2924-M	370	2	0	34	9.2
	6720-M	110	0	3	0	2.7
	Mean	331.7	7.5	3.2	6.3	3.1
Female	2C30-H	50	0	1	6	14
	4B67-H	1080	15	6	74	7.5
	4953-H	855	28	6	4	1.2
	IF70-H	5	2	0	0	0
	Mean	497.5	11.3	3.3	21	5.7

guttatus broodstock, supporting the usefulness of this reduced panel, for the assessment of multiple spawning events. The use of new genetic markers, such as SNPs, is an alternative for parentage testing in relevant aquaculture such as shrimp (Perez-Enriquez & Max-Aguilar, 2016), and oysters (Lapègue *et al*., 2014). Routine genotyping platforms are available; however, these types of platforms are not yet available for *L. guttatus*. Other techniques (*e.g.*, KASP, Taqman, HRM) are not economically feasible for more than 50 SNPs.

The reproductive pattern of males, most of them reproducing throughout the season and during consecutive days, has also been observed in the California yellowtail *Seriola lalandi* Valenciennes, 1833 (Smith *et al*., 2015). In wild *L. guttatus* females, asynchronous development of the gonads and partial spawning behavior has been described (Arellano-Martínez *et al*., 2001). The overrepresentation of females should be taken into account for hatchery management as an unbalanced family size that can lead to an increased inbreeding rate (Perez-Enriquez *et al*., 1999; García-Fernández *et al*., 2018).

Parentage assessment within a day of a spawning event by using the DNA extracted from fish embryonated eggs is recommended using a mechanical method rather than a chemical method as in other fish species [*e.g.*, gilthead seabream *Sparus aurata* (García-Fernández *et al*., 2018); zebrafish *Danio rerio* (Westerfield, 2007)]. However, the selection of embryonated eggs posterior to gastrula for DNA analysis is critical for PCR success, as similar results were reported for the gilthead seabream (García-Fernández *et al*., 2018).

Mutations and null alleles in microsatellites are a common phenomenon resulting in failed assignments (Ellegren, 2000). The mean mutation rate obtained in our study (10^{-2} per locus per generation) is higher than other fish species, such as the carp *Cyprinus carpio* with 10^{-4} (Yue *et al*., 2007), or various salmonids with 10^{-2}-10^{-5} (Shaikhaev & Zhivotovsky, 2014). Despite the high mutation rate, five high-variable microsatellites were enough to confidently determine, by direct exclusion, the parentage of progeny from a relatively small broodstock of the spotted rose snapper. For a larger broodstock, the number of genetic markers can be increased to minimize the non-exclusion probability (in the order of magnitude of 10^{-6} in the present study), using the remaining markers developed for the species (Table 1).

The estimation of the contribution of males and females of broodstocks kept in communal tanks is relevant for the implementation of selective breeding programs (García-Fernández *et al*., 2018). A more intensive and extended in time genotyping that gives a better genetic representation of the gene pool of the selected broodstock has been suggested for the red sea bream *Pagrus major* (Nugrohoa & Taniguchi, 2004) and the barramundi *Lates calcarifer* (Domingos *et al*., 2014). This information will also be important for the definition of the breeding goal, not only if the plan is focused on the improvement of reproductive traits, but also for other characteristics (growth, stress resistance, meat quality, others) (Gjedrem, 2012).

As an additional contribution, the genetic markers panel will also be useful for genetic studies in wild populations focused on their management in other lutjanid species.

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