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Isolation and characterization of thirteen polymorphic microsatellite markers from the bluestriped snappers *Lutjanus kasmira* and *L. Bengalensis*

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Abstract :

Thirteen polymorphic microsatellite loci were isolated and characterized from the reef fish *L. kasmira*. Polymorphism was studied in two populations from the South-West Indian Ocean, Seychelles and Tanzania. Polymorphism ranged from two to 16 alleles and loci are characterized by high variability. Two of these loci were characterized by highly significant heterozygote deficiencies for both populations and they should be avoided in future studies. Finally, eleven of these markers could be used to study population structure and connectivity of this species in a context of marine biodiversity conservation. Cross-amplification was tested for an other bluestriped snapper *Lutjanus bengalensis* and at least 5 of the 13 loci developed for *L. kasmira* could be used for this other bluestripped snapper.

Keywords : Microsatellite, *Lutjanus kasmira*, reef fish, Marine Protected Area

Introduction

Existing Marine Protected Areas (MPAs) in the West Indian Ocean (WIO) remain insufficient as a whole for the protection of marine reef diversity (Mora et al. 2006). In fact, for a real efficiency to preserve biodiversity, each MPA have to be integrated in a dense network of connected MPAs. In this context, genetic studies constitute an indirect approach to determine effective dispersal and delineate stock boundaries (Palumbi 2003) as it has recently been the case in the Hawaiian archipelago (Toonen et al. 2010). An effort is now needed to collect genetic data in the SWIO in order to provide an idea of marine species connectivity necessary for a regional management of marine biodiversity. That's why this study aims to develop microsatellite markers from the reef fish *Lutjanus kasmira* (Forsskal, 1775) to enable future population genetic studies. *L. kasmira* is a widespread Indo-pacific fish species, with a natural range from South Africa to Central Pacific (Allen & Talbot 1985). A recent phylogeographic study (Gaither et al. 2010) reveals no structure at the scale of the Indo-Pacific suggesting an important within-species connectivity. Microsatellites will strongly contribute to draw small-scale conclusions on connectivity.

Methods

Total genomic DNA was isolated from a single fish using Qiagen DNeasy spin columns and sent to GenoScreen, France (www.genoscreen.fr). 1 µg was used for the development of microsatellites libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as described in Malausa *et al.* (in press). Briefly, total DNA was enriched for AG, AC, AAC, AAG, AGG, ACG, ACAT and ATCT repeat motifs and subsequently amplified. PCR products were purified, quantified and GsFLX librairies were then carried out following manufacturer's protocols (Roche Diagnostics) and sequenced on a GsFLX-PTP. This technique allowed the identification of 3022 potential markers. The bioinformatics program QDD (Meglécz et al. in press) was used to filter for redundancy resulting in a final set of sequences for which it was able to design primers. Tetra-repeats were favored in order to reduce the presence of stutter bands and increase the chance of accurate scoring. Finally 70 validated sets of primers were designed and tested for amplification. Primer sets were discarded if they failed to amplify or lead to multiple fragments. At the end, 16 microsatellites were tested for polymorphism.

PCR amplifications were performed in 20 µL reactions containing 25 ng of template DNA, 1X reaction buffer (16 mM $(NH_4)2SO_4$, 67 mM Tris-HCl pH 8.8, 0.01% Tween-20), 1.5 mM MgCl2, 0.1 mM dNTP, 0.1 µM fluorescent primer, 0.l µM primer, and 1U Taq polymerase. The PCR cycling consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles: denaturation at 95°C for 30 seconds, annealing at the appropriate temperature (Table 1) for 30 seconds, and extension at 72°C for 1 minute and a final extension at 72°C for 20 minutes. Each microsatellite amplification was diluted with H_2O (1:20), mixed with Formamide and LIZ 500 size standard (Applied Biosystems). Fragments were separated using an Applied Biosystems 3100 DNA Analyzer. Alleles were scored using GeneMapper® v 4.0 (Applied Biosystems). GenAlEx v6 (Peakall & Smouse 2006) and Arlequin v 3.5.1.2 (Excoffier & Lischer 2010) were used to estimate diversities and expected and observed heterozygosities (He and Ho, respectively) and to test Hardy-Weinberg equilibrium (HWE).

Two out of 16 microsatellites studied were found to be monomorphic and one other gave low amplification success and were removed from the analysis. Table 1 summarizes the characteristics of the 13 primer pairs of polymorphic loci developed from the reef fish *L. kasmira* from two sample sets, one collected off the coast of Tanzania (6°48 S; 39°16 E) and the other in the Seychelles (6°48 S; 39°16 E) of, respectively, 16 and 21 individuals genotyped. The number of alleles ranged from 2 to 16 per locus with a mean of 6.8. Two significant linkage disequilibrium was observed for Tanzania population (Lk-26/Lk-49 and Lk-08/Lk-18; $p < 0.05$ but not significant after Bonferroni's correction (Rice 1989)) but not in Seychelles samples. Observed and expected heterozygosities (Ho and He) as well as fixation indices (Fis) are shown in Table 1. Three of the 13 loci failed to meet HWE for both populations, due to heterozygote deficiencies. Associated values of Fis were important (mean Fis > 0.3). Analysis using Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) indicated the presence of null alleles ($p < 0.05$) at some of these loci (see Table 1). Consequently, future studies might avoid to use the two Lk-31 and Lk-32 loci. Cross-amplification was tested for an other blue-striped snapper *Lutjanus bengalensis* (Bloch 1790) with 35 specimens sampled in Kenya (4°65 S; 39°38 E). Results are shown in Table 1. Amplification succeeded for the 13 loci and polymorphism was detected for 12 of the 13 loci (all except Lk-29 which is monomorphic). Null alleles were most frequently encountered for this species; consequently, it could limit at five the number of loci that could be used for *L. bengalensis*. High variability suggests that these markers could be used to study population structure and connectivity of both of these species which would be useful in a context of marine biodiversity conservation.

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Data Accessibility: DNA sequences: Genbank accessions JF346856- JF346867

Table 1 Characterization of 13 microsatellite loci for *Lutjanus kasmira* and *Lutjanus bengalensis*

with respectively Na, Ho and He, number of alleles, observed and expected heterozygosities at the population level; Fis, fixation index and test for deviation from Hardy–Weinberg expectations (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$); Null, the proportion of Null alleles as estimated by Micro-Checker.

Locus	Primer sequence (5′ to 3′)	Dye	Genbank accession number	Repeat motif	Range size (bp)	Annea-ling temp.	*L. kasmira* Tanzania (n =21)					*L. kasmira* Seychelles (n =16)					*L. bengalensis* Kenya (n =35)				
							Na	Ho	He	Fis	Null	Na	Ho	He	Fis	Null	Na	Ho	He	Fis	Null
Lk-05	TCCAGCTGTGAATGAGTGAGT GTGCTGTTTGATGATGGTGG	PET	JF346856	$(TGAA)_{13}$	165-237	60°	11	0.761	0.887	0.141	0,07	10	0.812	0.812	0.000	0.00	12	0.685	0.852	0.195***	0.11*
Lk-08	CTGAACCTCGTTCCCTCATC CTTGAGGCTTGCAAGGAAAT	FAM	JF346855	$(CTGC)_4$	167-191	60°	2	0.142	0.132	-0.077	-0.05	2	0.125	0.117	-0.06	-0.06	6	0.571	0.613	0.068	0.05
Lk-10	AACTTGAGCAGTGGAGCGAC GTTTCTGCAGTGACCTCCGT	VIC	JF346857	$(GTCTCT)_7$	181-241	55°	7	0.761	0.801	0.049**	0.00	6	0.750	0.736	-0.018	-0.00	7	0.400	0.744	0.462***	0.23*
Lk-18	TGCTGATGCTTAACTCAAAC ACAGAGAAGGTAAGCTGC	FAM	JF346858	$(ACAT)_4$	293-321	55°	4	0.285	0.290	0.015	0.03	2	0.125	0.117	-0.066	-0.05	8	0.628	0.760	0.173***	0.07
Lk-26	TGGTGGAGACGATATTGCAG AAGTGATTGAATAATTTGGCTTCTT	VIC	JF346859	$(ATAC)_4$	147-161	60°	4	0.238	0.530	0.551***	0.21*	4	0.312	0.322	0.030***	0.05	3	0.171	0.227	0.245*	0.11
Lk-27	TAGGAAGCAGGTGGGACTGT CTGCCAGTGACAAACAGGAG	NED	JF346860	$(TCCA)_{10}$	142-218	60°	14	0.761	0.910	0.163	0.08	14	0.812	0.908	0.105	0.05	14	0.800	0.891	0.102	0.06
Lk-29	TGGCCACATTCATTGTCTTT GGAAGGAATTTGGAGGGAAT	FAM	JF346861	$(GGCA)_4$	143-175	60°	4	0.333	0.422	0.211	0.07	4	0.250	0.599	0.583*	0.03	1	0.000	0.000	#N/A	-
Lk-30	AATGGATGGAAGTCACCTGG TCTTGTAAAGTGACTAAGTTTGAAATACG	PET	JF346862	$(TCTA)_{14}$	136-206	60°	11	0.761	0.869	0.123	0.06	14	0.937	0.900	-0.041	-0.02	15	0.342	0.591	0.420***	0.21*
Lk-31	AAGCAGACGATGACTGAAGACT GCACCATCGACACGTGATAC	NED	JF346863	$(AGAT)_{13}$	122-200	60°	16	0.523	0.883	0.406***	0.20*	12	0.375	0.892	0.579***	0.26*	11	0.342	0.795	0.569***	0.28*
Lk-32	TCCCATGTTTGTGCGTTAAG GTCAGGCCTGGATGTGAAGT	PET	JF346864	$(GCTAA)_4$	128-170	60°	6	0.238	0.708	0.664***	0.31*	7	0.312	0.789	0.603***	0.31*	7	0.314	0.665	0.527***	0.24*
Lk-42	AACTGATGAAGTGTTCAGATTTAGGA AGCTTTGCATGTCTGTTGTCA	VIC	JF346865	$(ATGTT)_5$	82-107	60°	2	0.047	0.336	0.858***	0.30*	3	0.250	0.314	0.204	0.08	4	0.285	0.300	0.050	-0.02
Lk-49	CTGATGTTGCCCAATAGGCT AAACAGTGAGACAGACAGCAAGA	FAM	JF346866	$(CTGG)_4$	117-129	60°	4	0.333	0.522	0.362	0.07	4	0.562	0.462	-0.21	-0.01	4	0.400	0.707	0.434**	0.22*
Lk-57	GATGGGTCTCTTCTTCGTGC CGCAACAACCTTCAGTCAGA	NED	JF346867	$(GCA)_5$	195-231	60°	4	0.428	0.427	-0.002	-0.04	5	0.500	0.570	0.123*	0.07	7	0.228	0.483	0.527***	0.18*

Footnote: All the individuals assayed have been successfully genotyped at all loci.