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Characterization of thirteen new polymorphic microsatellite markers from the honeycomb grouper *Epinephelus merra*

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Abstract:

Epinephelus merra (Serranidae) is the most common and widespread species of this genus, mostly found in reef habitats from South Africa to Central Pacific. Thirteen polymorphic microsatellite loci were isolated and characterized from this species. Polymorphism was studied in two localities from the Comoros archipelago—Mohéli and Moroni. Allelic diversity was 7.5, polymorphism ranging from 3 to 13 alleles. Overall heterozygosity was high with an average observed heterozygosity of 0.587. Three of these loci were characterised by heterozygote deficiencies for both populations. Finally, ten of the 13 developed primers could be used in future population genetics study of *E.merra* that might be useful in a context of marine biodiversity conservation.

Keywords: Microsatellite – *Epinephelus merra* – Reef fish – Indian ocean

1. Introduction

Groupers (Serranidae) are among the most abundant group of tropical marine fish (Randall & Heemstra 1991). A total of 332 species of groupers species inhabit the Indo-Pacific region (Randall & Heemstra 1991) with many being listed as critically endangered or threatened (Morris et al. 2000). The honeycomb grouper *Epinephelus merra* (Bloch 1793) is the most common and widespread species of this genus (Heemstra & Randall 1993). *E. merra* is mostly found in sheltered marine habitats such as lagoon and bays, from South Africa to Central Pacific (Randall & Heemstra 1991). *E. merra* is important in artisanal fisheries as food resource for many local communities (Heemstra & Randall 1993). Its wide-ranging distribution, its abundance and its fishing interest (and *in extenso* easy accessability of samples) make this species a good model for reef fish connectivity study. Existing microsatellite primers for groupers have been successfully tested on 16 grouper species (Ramirez et al. 2006) but when used on *E. merra* from West Indian Ocean, only two of the eight potential primer pairs gave satisfactory and reliable amplifications. This study aims so to characterize new microsatellite markers from the reef fish *E. merra* to enable future population genetic studies.

2. 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 2909 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, 15 tetranucleotide microsatellites were tested for polymorphism.

PCR amplifications were performed in 20 µL reactions containing 25 ng of template DNA, 1x reaction buffer, 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 nH_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 15 microsatellites studied were found to be monomorphic and were removed from the analysis. Table 1 summarizes the characteristics of the 13 primer pairs of polymorphic loci developed from the reef fish *E. merra*. Samples from Mohéli (12°24'10 S; 43°41'31 E) and Moroni (11°47'10 S; 43°14'55 E) - Comoros archipelago – were genetotype using the 13 developed primers. Upon the 60 samples collected (30 in each site) and analysed, the number of alleles ranged from 3 to 13 per locus with a mean of 7.5 (±3.4). 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. All deviations from HWE were

due to heterozygote deficiencies. These deficiencies might not be due to null alleles as amplifications succeed at more than 99.9%. Therefore we suggest to consider with caution these loci and especially the locus Epi-53 in future studies. Finally, ten of these 13 primers could be used in future population genetics study of *E.merra* and could be useful in a context of marine biodiversity conservation.

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References

Excoffier L, Lischer H (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res*, **10**, 564-567.

Heemstra PC, Randall JE (1993) Groupers of the World (Family Serranidae, Subfamily Epinephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO Fisheries Synopsis, No. 125, Vol16, 382pp.

Malausa T, Gilles A, Meglecz E, Blanquart H, Duthoy S, Costedoat C, Dubut V, Pech N, Castagnone-Sereno P, Delye C, Feau N, Frey P, Gauthier P, Guillemaud T, Hazard L, Le Corre V, Lung-Escarmant B, Male P, Ferreira S, Martin J (in press) High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Mol Ecol Res*.

Meglécz E, Costedoat C, Dubut V, Gilles A, Malausa T, Pech N, Martin J-F (in press) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics*, **26**, 403-404.

Morris A, Roberts C, Hawkins J (2000) The threatened status of groupers (Epinephelinae). *Biodiversity and Conservation*, **9**, 919-942.

Peakall R, Smouse P (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes*, **6**, 288-295.

Ramirez MA, Acevedo JP, Planas S, Carlin JL, Funk SM, McMillan WO (2006) New microsatellite resources for groupers (Serranidae). *Mol Ecol Notes*, **6**, 813-817.

Randall JE, Heemstra PC (1991) Revision of Indo-Pacific groupers (Perciformes: Serranidae: Epinephelinae), with descriptions of five new species. *Indo-Pacific Fishes*, **20**, 1-332.

Tables

Table 1 Characterization of 13 microsatellite loci for *Epinephelus merra*

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 (ns = non significant; * $p < 0.05$, *** $p < 0.01$)

Locus	Primer sequence (5′ to 3′)	Genbank accession number	Annealing temperature	Repeat motif	Range size (bp)	Mohéli (n = 30)					Moroni (n = 30)				
						Na	Ho	He	Fis	p	Na	Ho	He	Fis	p
EPI-04	CTGGTAGGACTGAGTGTCCTGA CCATTAACTGCCACTGGACC	JF429839	60°C	AGAC	90-134	7	0.433	0.767	0.435	***	10	0.733	0.805	0.089	ns
EPI-05	CATTCCTTCATGTTGTGAATGAC GGGATTATGTGCTGGCCTAT	JF429840	60°C	CTAT	99-153	11	0.666	0.863	0.228	ns	12	0.833	0.877	0.050	ns
EPI-07	AATTCCCTTGAGGCGTTCTT TTTATGCTGTCGGGTTCTCC	JF429841	60°C	GGAT	113-133	5	0.7	0.571	-0.225	ns	5	0.633	0.626	-0.011	ns
EPI-08	ATGAGAAGAGGGAAGCGGAT CTGCGTGGAGAAAGTGTGAA	JF429842	60°C	TGGA	111-127	6	0.666	0.689	0.033	ns	6	0.733	0.682	-0.074	ns
EPI-12	ATCCACCCATCTAGCTGACC AATGGATCGGAAATTGCTTG	JF429843	60°C	ATCC	135-145	3	0.4	0.439	0.089	ns	3	0.533	0.531	-0.004	ns
EPI-13	TGATTTGTCGAGGTAGGAGGA TTGACCAGCTGCTTTCTAAATG	JF429844	55°C	AATG	101-125	5	0.7	0.721	0.030	ns	5	0.566	0.561	-0.008	ns
EPI-16	CCCTGGGTGCTCTTAGTGTC TGCACGTTCAAAGTGGCTAA	JF429845	60°C	CTAT	131-157	6	0.5	0.680	0.265	*	6	0.266	0.706	0.622	***
EPI-27	TGTCCTCAGATAATGACTGAAACTG TTTGTACGAGCAATAATGGCA	JF429846	60°C	GATA	119-173	12	0.666	0.848	0.214	ns	13	0.666	0.873	0.237	ns
EPI-35	GGTGCTTATTTGTGCCAGGT AGCCATTCCTCAGCTCATTG	JF429847	60°C	ATGG	142-157	3	0.333	0.412	0.192	ns	3	0.466	0.586	0.204	ns
EPI-48	GGCCTACTGCAGAAGCTGTT AGCTACTTGCTCGGACCACA	JF429848	58°C	GTCT	168-196	7	0.633	0.783	0.192	**	6	0.566	0.732	0.226	ns
EPI-49	TTTGTCATGCAGGATTGTCC CAAACTAGAGTATACCAGTCTGCCA	JF429849	60°C	ATCT	131-198	12	0.866	0.883	0.018	ns	13	0.8	0.866	0.076	ns
EPI-50	TCTCCTTGACTTCCCTGCAC GCCTCATTTATTATTCTCTGCATGT	JF429850	58°C	CAGA	170-198	8	0.5	0.773	0.353	**	6	0.366	0.776	0.527	**
EPI-53	ACTGCAGCTCCTTGGTGACT ACATTATGATTCTTCCTGTAGGG	JF429851	60°C	ATCT	180-240	11	0.566	0.886	0.360	***	11	0.466	0.874	0.466	***