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PRIMER NOTE

More polymorphic microsatellite markers in the European sea bass (*Dicentrarchus labrax* L.)

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Abstract

We provide details of five microsatellite loci screened in 163 individual sea bass. Large numbers of alleles were observed at three loci (20–25) and heterozygosities ranged from 0.52 to 0.86. These loci should prove useful for population genetic studies and for the pedigree analysis and genetic management of this species in aquaculture.

Keywords: *Dicentrarchus labrax*, microsatellites, seabass

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Dicentrarchus labrax is now an economically important farmed species in Europe. Sustainable aquaculture practices require the genetic management of this species, and it is therefore important to complement and expand the number of polymorphic genetic markers, especially microsatellites, that already exist (García de León *et al*. 1995, 1997, 1998; Castilho & McAndrew 1998).

The population genetic structure of seabass suggests that there are significant population subdivisions in both Atlantic and Mediterranean parts of its range (Bahri-Sfar *et al*. 2000; Castilho & McAndrew 1997; García de León *et al*. 1997; Naciri *et al*. 1999) possibly maintained by strong natal spawning ground homing (R. Castilho & B.J. McAndrew, submitted). Differential selection on populations exposed to marine and less saline lagoon habitats has also been observed (Lemaire *et al*. 2000). Further work in these areas will substantially benefit from the availability of more markers. Here we describe the development of five microsatellite markers for *D. labrax* that will be of potential use in studies of genetic diversity, population structure and the genetic management in this commercially important species.

Five microsatellite loci were isolated from sea bass size-selected genomic library as described in Castilho & McAndrew (1998). Plasmid DNA was extracted from 5 mL overnight cultures of *Escherichia coli* in Luria–Bertani medium using Qiaprep spin miniprep kit (Qiagen) according to the manufacturer's instructions. The purified positive clones were sequenced using the ABI PRISM dye terminator cycle sequencing ready reaction kit on an ABI PRISM 377 DNA sequencer (PE Applied Biosystems). Oligonucleotide primers were designed with the aid of the PRIMER program. Variability of each locus was examined in a total of 163 individuals taken from three populations in Portugal, Greece and Turkey. Amplifications were performed in a 10-μL polymerase chain reaction (PCR) mixture containing 20 ng genomic DNA, 1 μL PCR buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl), 1.5 mM $MgCl_2$, 0.24 units Amplitaq Gold (Applied Biosystems), 200 μM of each dNTP (Sigma) and 6 pmol of each primer. The reverse primers were 5′ end-labelled with [γ-^{32}P] ATP (specific activity = 4500 ci/mmol, ICN Chemicals; 37 kBq/10 pmol primer) using T4 polynucleotide kinase (Gene Sciences) according to standard protocols (Sambrook *et al*. 1989).

The samples were amplified on a DNA Thermal Cycler (model PE 9700, Applied Biosystems) according to the following protocol: 10 min initial denaturation at 95 °C (hot start), followed by 30 cycles of 30 s at 94 °C, 30 s at the corresponding primer annealing temperature and 30 s at 72 °C. Before cooling to 4 °C, the products were incubated 7 min at 72 °C. The polymorphism was screened in a polyacrylamide sequencing gel using an M13mp18 (Bst premixed sequencing kit Bio-Rad) sequencing ladder as size marker. PCR products were visualized by exposure to X-ray film (Kodak XAR-5).

PCR primers, optimal PCR conditions and assessment of variability for five microsatellite loci are shown in Table 1. The 5 loci all showed distinct allelic variation ranging from 7 to 25 alleles per locus (average ± SD = 16.4 ± 8.3). Mean observed heterozygosity (0.73) is lower than mean

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Table 1 GenBank Accession nos, repeat motif, primer sequences, annealing temperature, number of individuals, size range, number of alleles, observed heterozygosity (H_O) and expected heterozygosity (H_E) for five microsatellite loci isolated from the seabass (*Dicentrarchus labrax*)

Locus	GenBank / EMBL Accession no.	Repeat motif	Primer sequence (5′ → 3′)	T_a (°C)	No. of indiv.	Size range (bp)	No. of alleles	H_O	H_E
Dla-12	AY125920	$(AC)_{30}$	F′-GTATGTTGCCAGAGCCAAGC R′-CAGACAAACTGTATGCCTGC	56	163	245–297	22	0.8037	0.9033
Dla-14	AY125921	$(CA)_3A_3(CA)_{14}$	F′-CAAAGACATCACTGTAGAGG R′-AATCAGACACTCAATCACCC	53	163	177–193	8	0.6380	0.6836
Dla-20	AY125922	$(CA)_{22}(GA)_{10}$	F′-TGCTGTCTTGACCGTTAGCC R′-TGAGTGAGTTTGTCCTGCCG	57	162	275–343	25	0.8210	0.9360
Dla-22	AY125923	$(TG)_7CG(TG)_{26}$	F′-GTTGTGCGTCTGTTGTCACC R′-ATTTGCTCCTGATTGCCCCG	57	162	240–278	20	0.8580	0.9218
Dla-47	AY125924	$(CA)_{11}$	F′-CGGATGAAACCAGTAGTTCC R′-AGTTCTTAGACAGAGCGAGG	55	157	283–296	7	0.5223	0.6014

expected heterozygosity (0.81), and there is a trend to heterozygote deficit at all five loci. This could be due to a Wahlund effect considering the geographical sources of the individuals, that is, we could have created an apparent heterozygote deficit by sampling structured subpopulations across allele frequencies at which these loci are differentiated. However, the presence of null alleles cannot be discounted.

In summary, these five microsatellite loci exhibited a large number of alleles per locus and high heterozygosity. This suggests that, in combination with previously published primers (García de León *et al*. 1995; Castilho & McAndrew 1998), these five novel microsatellite loci will be useful for further population genetic studies of wild populations. The markers would also be helpful in the management and improvement of farmed strains and monitoring possible interactions between farmed and wild populations.

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