

# Isolation and characterization of polymorphic microsatellite markers for peacock wrasse (*Symphodus tinca*)

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## Abstract

Eight polymorphic microsatellite loci were isolated and characterized for the peacock wrasse (*Symphodus tinca*), a labrid fish inhabiting the Mediterranean and Black seas. Characterization of 35 individuals from the western Mediterranean indicated a relatively high allelic diversity (mean = 12.4, range 9–17), and observed heterozygosity ranging from 0.65 to 0.91. We found no evidence of linkage disequilibrium between pairs of loci. Two loci showed significant departure from Hardy–Weinberg equilibrium. These polymorphic markers can be useful in most basic population genetic applications.

Keywords: enrichment, microsatellites, *Symphodus tinca*, wrasse

The peacock wrasse (*Symphodus tinca*) is a marine fish of the family Labridae attaining a maximum length up to 35 cm. This species occurs in the eastern Atlantic and the Mediterranean and Black seas (Quignard & Pras 1986). It is not subject to targeted food fisheries. However, it is not rare to find it as a by-catch of fisheries focused on other species and its catches contribute significantly to recreational angling and spearfishing activities (Gordoa et al. 2000).

The peacock wrasse is found mainly on reefs and shallow coastal areas. Males are territorial and conspicuous male nuptial colouration and courtship have been observed (Costello 1991). The spawning season begins in April and ends in late June (Warner & Lejeune 1985). Planktonic larval duration ranges from 9 to 13 days and settlement takes place from early May onwards (Raventos & Macpherson 2001). Genetic studies are scarce and have relied mainly on mitochondrial DNA (mtDNA) to infer phylogenetic relationships within Labrids (Hanel et al. 2002). With the aim of estimating genetic variability at the nuclear level, here we report eight new polymorphic microsatellite DNA loci for the peacock wrasse.

An enriched genomic library was constructed following the methods of Glenn (2000) available at [www.uga.edu/](http://www.uga.edu/)

[srel/DNA\\_Lab/protocols.htm](http://srel/DNA_Lab/protocols.htm), with modifications to the protocol as detailed in the succeeding text. Approximately 10 µg of high molecular weight DNA of 10 individuals from Blanes (Western Mediterranean) was isolated by phenol–chloroform extraction followed by ethanol precipitation (Sambrook et al. 1989). DNA extractions were performed from pectoral fin tissue, RNase treatment was applied where required and recovered DNA was diluted to ~200 ng/µL final concentration. The genomic DNA was simultaneously digested with RsaI restriction enzyme and ligated to double-stranded linker-adapted primers (Hamilton et al. 1999). Restricted-ligated DNA fragments were amplified with linker-adapted primers and hybridized with a biotinylated probe mixture consisting of (GT)<sub>10</sub> and (GATA)<sub>10</sub> (CT)<sub>10</sub> at 10 µm each. DNA fragments with repetitive sequences were selectively captured by streptavidin-coated Dynabeads (Oxoid) and separated by a magnetic field. DNA was eluted in dH<sub>2</sub>O from the bead probes and concentrated by vacuum centrifugation to a final concentration of ~100 ng/µL. DNA was then reamplified by polymerase chain reaction (PCR) using linker-adapted primers. Subsequent ligation of enriched/recovered DNA into a cloning vector was carried out using a TOPO TA cloning kit (Invitrogen) following the manufacturer's protocol.

More than 180 positive clones were obtained. All clones were sequenced and checked for inserts using ABI PRISM BigDye Terminator Cycle kit (Applied Biosystems) and

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Table 1 Characterization of eight peacock wrasse (*Symphodus tinca*) microsatellite loci. GenBank Accession no., locus name, repeat motif, fluorescent dye-primer sequence, number of alleles, allele size range,  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity under Hardy–Weinberg equilibrium;  $F_{IS}$ , inbreeding coefficient, \* $P < 0.05$

GenBank Accession no.	Locus	Repeat motif	Primer sequences (5'–3')	No. of alleles	Allele size (bp)	$H_O$	$H_E$	$F_{IS}$
DQ324538	Stin155	(GA) <sub>11</sub>	F: FAM-GAGGCAAACAAGTATTTC R: AGGGACTGTGTCTCTTGATG	11	138–168	0.8611	0.8798	0.054
DQ324537	Stin287	(AC) <sub>6</sub> GC(AC) <sub>9</sub>	F: FAM-AGCAGGTTCAATAAACACA R: GGGTGCTCAAGGTAATTGT	9	275–297	0.8055	0.8047	0.001
DQ324543	Stin368	(AC) <sub>22</sub>	F: FAM-TCTGGCAGCCTTAGTCCTC R: GAGCGTCTTCAGAAGGACA	17	336–368	0.7500	0.8873	0.247*
DQ324540	Stin143	(AC) <sub>4</sub> G(AC) <sub>14</sub>	F: HEX-TGCAAGCAGAGTCTCAAACCC R: ATTTAACAGCTTGCTTGG	11	120–141	0.9166	0.8924	–0.027
DQ324542	Stin222	(TG) <sub>9</sub> C(TG) <sub>17</sub>	F: HEX-CCCCTGATAGATGAGGAG R: GGTGCTCCTGTGATATCT	11	206–231	0.7777	0.8321	0.159
DQ324539	Stin336	(CA) <sub>36</sub>	F: HEX-GCTGGAAGAATAGAACATTC R: TTTTGTGCAAGCTTTTAGT	13	321–365	0.7714	0.8567	0.191
DQ324541	Stin245	(GATA) <sub>30</sub>	F: NED-TAGCCCCGCCCTAAAAA R: TGCTACTGGATGGCGCTGAA	12	218–314	0.8463	0.8961	0.084
DQ324536	Stin138	(C) <sub>16</sub> (CA) <sub>11</sub>	F: NED-GCGTTTTTACGTCATGTTTT R: AGGTATGTGTCCGCTTTT	15	127–165	0.6571	0.9155	0.424*

resolved on an ABI 3100 Genetic Analyser (Applied Biosystems). PCR primers were designed using oligo 6.4 software. PCRs were performed in 25  $\mu$ L total volume, which includes 50 ng of DNA, 1 mm of  $MgCl_2$ , 0.75  $\mu$ M of forward and reverse universal M13 primers, 200  $\mu$ M dNTP's, 1 $\times$ reaction buffer [75 mm Tris-Hcl, 20 mm  $(NH_4)_2SO_4$ ] and 0.5 U Taq polymerase (BIOTAQ). Reaction conditions were as follows: an initial denaturation step of 5 min at 95 °C, 30 cycles consisting of 30 s at 92 °C, 30 s at 55 °C annealing temperature and 30 s at 72 °C. Individuals were genotyped by assessing allele size on an ABI 3100 Genetic Analyser (Applied Biosystems) using forward primers labelled with FAM, HEX (Sigma) and NED (Applied Biosystems). Allele scoring was carried out using genemapper version 3.5 software (Applied Biosystems).

Microsatellite variability was tested in 35 individuals from Blanes. Observed and expected heterozygosities as well as deviations from Hardy–Weinberg expectations were calculated using arlequin (Schneider et al. 2000). The number of alleles per locus and allele size range were estimated using fstat version 2.9. (Goudet 1995). All loci were polymorphic, the number of alleles per locus, expected and observed heterozygosities are listed in Table 1. There was no evidence of linkage disequilibrium between locus pairs. Two loci showed significant departures from Hardy–Weinberg equilibrium. This could be due to segregation of null alleles or population subdivision. With the present data set, we cannot distinguish conclusively between these alternatives, but the likeliest explanation is presence of null alleles segregating at high frequencies.

Nonetheless, these results suggest that most of the loci characterized could be useful for most population genetics applications.

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