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PRIMER NOTE

# Isolation of polymorphic microsatellite markers for the demersal fish *Helicolenus dactylopterus* (Dela Roche 1809)

M. A. ABOIM,*‡ A. D. ROGERS,*† G. M. MENEZES,‡ R. MAGGIONI* and C. V. M. PEARSON*
*School of Ocean and Earth Sciences, Southampton University, Southampton Oceanography Centre, European Way, Southampton SO14 3ZH, UK, ‡Departamento de Oceanografia e Pescas da Universidade dos Açores, Cais de Sta Cruz, 9900 Horta, Açores, Portugal*

## Abstract

**Six microsatellite loci were identified for the demersal deep-sea fish *Helicolenus dactylopterus*. All loci were highly polymorphic (5–21 alleles per locus). Observed heterozygosities were from 0.378 to 0.868, while the expected heterozygosities ranged from 0.529 to 0.925. Multiplex PCR reactions were optimized. Microsatellite markers were developed for analysis of genetic structure including identification of stocks and migration patterns. The resulting data will be used to help in the establishment of scientifically based fisheries management for this species. Departures from the expected Hardy–Weinberg equilibrium were observed in three loci, and are likely to be a consequence of population structuring across the Azorean islands.**

*Keywords*: Azores, enriched library, *Helicolenus dactylopterus*, microsatellites, multiplex PCR

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The demersal long-line fishery directed towards *Pagellus bogaraveo* is one of the main marine biological resources in the Azores archipelago. Several other species represent significant landing values in this fishery (Menezes *et al*. 1997) among the most important of which is the bluemouth, *Helicolenus dactylopterus* (Dela Roche 1809).

*H. dactylopterus* is a scorpion fish of the family Sebastidae. It is commonly found between 400 and 600 m depths along the edge of the continental shelf and upper continental slope of the eastern Atlantic (from Norway to the Gulf of Guinea), and the western Atlantic (from Canada to Brazil) and also, on the slopes of the Macaronesian islands (Azores, Madeira, Canaries and Cape Verde), mid Atlantic Ridge and associated seamounts. This species is known to have internal fertilization and was thought to have a reproductive strategy involving either viviparity or oviparity (Krefft 1961). Free-floating planktonic eggs have been documented, however, and no developed embryos have been observed in the uterus of female fish (Muñoz *et al*. 1999).

Correspondence: Maria Ana Aboim. School of Ocean and Earth Sciences, Southampton University, Southampton Oceanography Centre, European Way, Southampton SO14 3ZH, UK. Fax: +44 (0) 2 8059 3052; email: maa1@soc.soton.ac.uk
†Present address: British Antarctic Survey, High Cross, Madingley Road, Cambridge, CB3 0ET, UK

These aspects of life-history, together with observations that indicate adults are sedentary over periods of years (Menezes, personal observation), lead to the expectation of low levels of dispersal in this species. Therefore populations of *H. dactylopterus* may show marked genetic differentiation within the North Atlantic area.

Microsatellites have been developed as genetic markers for the identification of population structure and fisheries stocks for management purposes. In the present work microsatellites were identified and assessed for variation in populations of *H. dactylopterus* from the Azores Islands.

Specimens were collected throughout the Azores Archipelago and adjacent seamounts. Genomic DNA from muscle tissue or liver was isolated from 100 individuals using a phenol-chloroform extraction protocol (Sambrook *et al*. 1989). An enriched library technique based on Kandpal *et al*. (1994) with modifications by Morgan *et al*. (1999) was used to isolate microsatellite loci. Extractions of 20 individuals were combined and digested using the *Mbo*I restriction enzyme. 200–500 bp fragments were purified from agarose gels, ligated with T4-ligase to *Mbo*I adaptor molecules, and amplified by polymerase chain reaction (PCR). Purified PCR products were hybridized with a synthetic $(CA)_n$ probe labelled with biotin, and separated with streptavidin–coated magnetic beads. DNA fragments rich

**Table 1** *Helicolenus dactylopterus*. Characterization of six microsatellite loci

| Locus | GenBank Accession No. | Primers (5′–3′) — fluorescent dye | Repeat Motif | Size Range | No. of alleles | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|
| Hd 008 | AY123151 | F-GCATGTGATGACCTTTGACC<br>R-GTTACAGCGGCAAGAAACC-6FAM | $(CA)_6CCCATGTA(CA)_8CCTATGTA(CA)_{14}$ | 204–249 | 18 | 0.800* ($n$ = 25) | 0.888 |
| Hd 044 | AY123152 | F-AATGGGCTGAACTGTCCTTG<br>R-CTCTGACTGCTTCCTGGGTC-HEX | $(GT)_9$ | 183–192 | 6 | 0.378** ($n$ = 37) | 0.529 |
| Hd 063 | AY123153 | F-GGCTCTGTCTATCTCTCGCC<br>R-TTCTGAGTTCCCAAACACCC-NED | $(GTGTGTGTT)_4(GT)_4$ | 208–235 | 5 | 0.600 ($n$ = 40) | 0.659 |
| Hd 092 | AY123154 | F-TGATGCAGTGGTGGAGAGAG<br>R-ACCTTCTATCTGACGCGAGG-6FAM | $(CA)_{21}$ | 166–206 | 20 | 0.868 ($n$ = 38) | 0.925 |
| Hd 095 | AY123155 | F-TTGGCTTTTTGTCGAGGG<br>R-GCTAACATCAGCACGAATGG-NED | $(CA)_{12}$ | 115–191 | 21 | 0.684* ($n$ = 38) | 0.880 |
| Hd 106 | AY123156 | F-AGCTTGGGCTGAAAGATGG<br>R-TGGCAGCAGAGATGAACG-6FAM | $(GT)_5TT(GT)_{13}$ | 135–183 | 18 | 0.641 ($n$ = 39) | 0.785 |

$n$ = sample size; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; * = $H_O$ significantly different from $H_E$ < $P$ = 0.05; ** = $H_O$ significantly different from $H_E$ < $P$ = 0.01.

in $(CA)_n$ repeats were ligated into pGEM-T plasmids to transform *E. coli* (JM109 strain). Around 200 colonies were successfully transformed and 33 were positive for $(CA)_n$ repeats. 25 clones were sequenced using an ABI 377 sequencer using M13 universal primers and Perkin-Elmer ABI Big Dye Terminator sequencing kits, according the manufacturers instructions.

Primers were designed using the programme PRIMER 3 (Rozen & Skaletsky 1998) for 6 microsatellites. All loci showed reliable amplification in multiplex PCRs with fluorescently labelled primers at an annealing temperature of 58 °C. Characteristics of the primers and respective microsatellite loci are given in Table 1.

PCRs were performed in a MWG-Biotech Primus 96 Plus machine, consisting of an initial denaturation step of 4 min at 94 °C, followed by 30 cycles of 94 °C-60 s, 58 °C-45 s, 72 °C-60 s and an extension step of 72 °C for 20 min. Ten μL multiplex (with two or four primers) reactions contained approximately 20 ng of DNA; 1 μL 10× Qiagen buffer (Tris-HCl pH 7.8, KCl, 1.5 mM $MgCl_2$); 2 μL Qiagen 'Q solution'; 0.9 μL DNTP's mix (Perkin Elmer); 0.75 U *Taq* polymerase (Qiagen); 7 pmol of each primer; 0.1 μL extra dATP and chemical water. Multiplex 1 (with two primers) contained Hd092 and Hd095 loci in the same PCR reaction, while multiplex 2 (with four primers) contained the remained loci.

All six microsatellite loci were scored for 40 individuals from the Azores archipelago showing high polymorphism with 5–21 alleles per locus. One of the loci (Hd 044) showed significant deviation from the expected genotype frequency under Hardy–Weinberg equilibrium at $P < 0.01$ and two loci at $P < 0.05$ (Hd008, Hd095) (Fisher's Exact test; ARLEQUIN version 1.1, Schneider *et al*. 1997).

The departures from the Hardy-Weinberg expected genotype frequencies can result from null alleles, selection or nonrandom mating within the chosen sample. Subdivision of the Azorean metapopulation into demes around the three island groups and seamounts are likely to be responsible for the observed significant deviations from Hardy-Weinberg expected frequencies.

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