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TECHNICAL NOTE

Development of eleven microsatellite loci in the deep-sea black scabbardfish (*Aphanopus carbo*)

Halvor Knutsen · Diana Catarino · Hanne Sannæs · Sergio Stefanni

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Abstract We developed 11 microsatellite loci primers in the bathypelagic black scabbardfish (*Aphanopus carbo*), a novelty for deep-sea fishes. All markers were obtained from partial genomic DNA libraries enriched for tetranucleotide repeats and characterized in 50 unrelated individuals from one putative population. The number of alleles ranged from 5 to 40, with an average of 19.3 per locus, and the observed heterozygosity ranged from 0.387 to 0.961 (average 0.749). Cross-amplification in another closely related commercially exploited deep-sea species intermediate scabbardfish (*Aphanopus intermedius*) resolved 11 polymorphic loci.

Keywords *Aphanopus carbo* · *Aphanopus intermedius* · Microsatellite primers · Polymorphisms

The black scabbardfish (*Aphanopus carbo*) is a bathypelagic species inhabiting temperate-cold Atlantic waters and mostly found within the depth range between 200 and 1,800 m (Tucker 1956; Martins et al. 1987). The species has an extremely elongated body whose color is coppery black with iridescent tint. Scabbardfish migrate to mid-water at night and feed on crustaceans, cephalopods and fishes (Parin 1986). Adults mature at lengths of 80–85 cm (Figueiredo et al. 2003) and so far the spawning is only known to happen off Madeira during September to February. Eggs and larvae are pelagic (Parin 1986).

Recently, it was reported that *A. carbo* and the closely related species *A. intermedius* live in sympatry in the Azores, Madeira, Canaries and north western coast of Africa (Stefanni and Knutsen 2007; Stefanni et al. 2009). These two species have overlapping morphological characters and the use of genetics is currently the only safe way to differentiate the two species (Stefanni et al. 2009). The scabbardfish is valuable in fish markets of Portugal (Martins et al. 1994; FAO 2002; Bordalo-Machado and Figueiredo 2009) and more recently commercialized also in the United Kingdom, Ireland, northern France and Spain. The current knowledge about population structure, as a basis for management, is lacking for both species. Hence, the overall result is that these two species are most likely mixed up, resulting in confused commercial exploitation. Therefore, there is an urgent need for a better understanding of population structuring for these two species. Here we present 11 microsatellite loci developed for *A. carbo* that also cross amplify with *A. intermedius* and are thus usable as a method for detecting potential population structure in both species.

We employed the company GIS (Genetic Identification Service Inc.) for the development of tetra-repeat loci. An enriched subgenomic library was constructed as described in Meredith and May (2002) and Schwartz and May (2004), and four libraries were screened for the microsatellite motifs (AAAC)n, (CATC)n (TACA)n and (TAGA)n. A total of 100 clones were sequenced and 19 primer pairs were designed. Eleven of these were found to be polymorphic and reliably amplified, and all further tests were restricted to these eleven loci.

Population screening was conducted by analysing of 50 individuals, all genetically identified as black scabbardfish

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Table 1 Primer sequences and characteristics of 11 black scabbardfish (*Aphanpous carbo*) microsatellite loci

Locus	GenBank acc. no	T_a (°C)	Repeat motif	Primer sequences (5′-3′)	Size range (bp)	N_A	H_E	H_O	F_{IS}	*P*-value
AcA105	xxx	56	$(AAAC)_{14}$	F: CACCAAGTTTCACCAGAATC R: TGATGGAGGAGAGTCAGTGT	155–183	7	0.753	0.735	0.025	0.594
AcA109	xxx	57	$(AAAC)_6$	F: TAACGCTGACTGTTTCACTG R: CCAGACATACGAGGTTTGAC	245–265	5	0.337	0.387	−0.152	1
AcA112	xxx	58	$(AAAC)_6$	F: AGGGTCAGTGTTTCACAGATAG R: GAATCACCAGCAGAGTTCAG	302–330	7	0.769	0.733	0.048	0.549
AcB3	xxx	58	$(CCAT)_{18}(ACAT)_9$	F: CGGCACATAGATGACATGA R: CGTGTCCCTCCTCATATTG	225–278	29	0.963	0.961	0.002	0.696
AcB103	xxx	55	$(CCAT)_{32}$	F: CAGCCCATTGTGTTTATC R: AGGATGAAGGTATAGAGAAATG	73–229	30	0.949	0.838	0.120	0.037*
AcB118a	xxx	56	$(CA)_{10}$	F: CCACCTACCAAATTATCCA R: AGAATTGTACGGGCTGAGTC	143–199	16	0.717	0.729	−0.016	0.7211
AcC101	xxx	58	$(TACA)_{16}$	F: GGCAGATTTTGAGATTTTCAAC R: AGCCACTGAACTGAATAACTGC	206–298	17	0.882	0.775	0.122	0.010*
AcC106	xxx	57	$(GACA)_5(TACA)_6(GACA)_2$	F: AGACCCCTGAAGAGTGTTTTG R: AGAATTTGCCTGAGCTGGTAC	152–208	10	0.767	0.416	0.460	0.0001***
AcD7	xxx	57	$(TAGA)_{18}$	F: ATGGTGTGTGTCAGTGTATGTG R: TTGTCTGGTCCGAGTGTG	173–249	17	0.923	0.918	0.005	0.556
AcD105	xxx	54	$(CTAT)_{13}$	F: GCTGATTGGAAGCTGATG R: TGAATGACACAGACTGACAGA	229–373	34	0.966	0.911	0.057	0.150
AcD109b	xxx	58	$(CTAT)_{27}$	F: TGCAGGGATGAACATCTTACTC R: TGAACCATAAAGCATTAGCACA	170–462	40	0.977	0.837	0.145	0.1064

Size range of fragments (bp), number of alleles (N_a), expected (H_E) and observed (H_O) heterozygosity and deviation from Hardy–Weinberg expectations (F_{IS}), are based on a sample of 50 individuals. Uncorrected *P*-values for two-sided tests, * $P < 0.05$, *** $P < 0.001$

using the method of Stefanni et al. (2009), caught on the Mid Atlantic Ridge (38.30 N; 28.40 W) close to the Azores. Individuals identified as *A. intermedius* were utilized in testing cross-amplification of the developed primer loci. Genomic DNA was isolated using Viogene Blood and Tissue Genomic DNA Extraction Miniprep System (Viogene Inc.) according to manufacturer's protocol. PCR amplifications were carried out in 10 µl reaction volumes on Bio-Rad MYCycler, with fluorescently (CY-5) 5′-tagged forward primers (Sigma). The standard reaction composition included 1 µl of template DNA, corresponding to 20–40 ng, 10 × 15 mM $MgCl_2$ PCR buffer, 0.4 mM dNTPs, 0.125 mM of forward and reverse primers (Sigma) and 0.06 units μl^{-1} of Taq DNA polymerase (Qiagen Inc.). Dilutions were done using Eppendorf Molecular Biology Grade Water. Thermal cycling conditions were as follows: An initial denaturation step at 94°C for 5 min, followed by 30 cycles of 95°C denaturation, annealing at specific temperature (cf. Table 1) and 72°C synthesis, each for 30 s. A final elongation step at 72°C for 15 min completed the amplification.

Allele sizes and genotypes were determined by fragment analysis using Beckman Coulter CEQ 8000 automated sequencer and included software (CEQ8000 Genetic Analysis System, version 8.0). We tested the loci for all individuals to assess gene diversity and evidence for linkage disequilibrium or deviation from Hardy–Weinberg expectations. Gene diversity was estimated with GDA (Lewis and Zaykin 2001); F_{IS} was estimated and tested using the probability tests within GENEPOP on the web (http://wbiomed.curtin.edu.au/genepop/). The software MICROCHECKER (Van Oosterhout et al. 2004) was used to investigate the potential presence of null alleles or other technical artifacts. Three loci showed signs of Hardy–Weinberg deviations. Two of these loci were barely significant (uncorrected *P*-values: AcB103, $P = 0.037$; AcC101, $P = 0.010$, cf. Table 1). The third locus, AcC106, however showed clear significant deficiency of heterozygotes ($P < 0.0001$; cf. Table 1), and was estimated to contain 29% null alleles. We found indications of null-alleles for the two firstly mentioned loci AcB103, AcC101 (4–5%), but this may also be due to coincidence by using too few individuals. We also tested for presence of linkage disequilibrium (LD) between pairs of loci using GENEPOP, but no evidence for LD was detected. Finally, we tested all loci for cross species amplification on 10 individuals of the closely related species *Aphanopus intermedius* resulting in 11 useful microsatellite DNA loci for this species also (Table 2). Although all loci amplified with similar size ranges, several rare or unique alleles were identified in the cross amplification.

Table 2 PCR cross-amplification of microsatellite loci in *Aphanopus intermedius* using the 11 primers developed for *A. carbo* (cf. Table 1)

Locus	*A. intermedius* ($n = 10$)				
	Size range (bp)	N_A	H_E	H_O	F_{IS}
AcA105	159–195	8	0.799	0.866	−0.1166
AcA109	245–257	5	0.518	0.500	0.0370
AcA112	306–342	9	0.804	0.812	−0.0104
AcB3	242–310	12	0.931	0.833	0.1093
AcB103	61–221	16	0.951	0.620	0.3621
AcB118a	149–191	10	0.812	0.875	−0.0797
AcC101	206–278	11	0.891	0.857	0.0400
AcC106	172–200	8	0.795	0.667	0.1667
AcD7	181–225	10	0.789	0.687	0.1316
AcD105	229–389	19	0.975	1	−0.0263
AcD109	178–438	17	0.966	0.769	0.2105

All 11 primers successfully amplified and were found to be polymorphic among ten individuals ($n = 10$) Size range (in base pairs, bp) refers to specific alleles, N_A is total number of alleles, H_E refers to expected and H_O to observed heterozygosities, and F_{IS} to deviation from Hardy–Weinberg expectations (HWE). As only 10 individuals were used, the HW estimates is only indicative of possible deviations

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