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TECHNICAL NOTE

Development of twelve microsatellite loci in the corkwing wrasse (*Symphodus melops*)

Halvor Knutsen

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Abstract We developed 12 microsatellite loci primers in the corkwing wrasse (*Symphodus melops*). All markers were obtained from partial genomic DNA libraries enriched for tetranucleotide repeats and characterized in 32 unrelated individuals from one putative population. The number of alleles ranged from 5 to 18, with an average of 8.6 per locus, and the observed heterozygosity ranged from 0.464 to 0.969 (average 0.697). Cross-amplification in two closely related commercially exploited species, the ballian wrasse (*Labrus bergylta*) and the goldsinny wrasse (*Ctenolabrus rupestris*), successfully resolved four loci of which two were polymorphic and two where monomorphic.

Keywords *Symphodus melops* · *Ctenolabrus rupestris* · *Labrus bergylta* · Microsatellite primers · Polymorphisms

The corkwing wrasse (*Symphodus melops*) belongs to the family Labridae which is the third lagest family of marine fish with 580 species in 82 genera (Pareti and Randall 2000). *S. melops* is a rocky shore species inhabiting temperate-cold Atlantic waters from Norway to Morocco and the Azores. It may reach an age of 9 years and about 28 cm in length (Quignard and Pras 1986). The species coloration is very variable; with the ground color of the male being greenish or blue while females are brownish to yellowish (Muus and Nielsen 1999). The diet mostly consists of mollusks, hydroids, bryozoans, worms and various crustaceans. Males grow faster than females (Quignard and Pras 1986). The species is most commonly found in the upper 30 m of the water column and is believed to be non-migratory with a territorial behaviour. Males build seaweed nest that they gard among rocks or in crevices, and ripe females show short ovipositor during summer. Sex reversal is sometimes observed (Quignard and Pras 1986).

The Labribes (species like *S. melops*, *Ctenolabrus rupestris* and *labrus bergylta*) is increasingly being exploited commercially by the salmon industry, to remove lice from salmon (*Salmo salar*) and have over the last decade become a commercially important resource (Treasurer 2002). The current knowledge about population structure, as a basis for management, is lacking for all these species. Therefore, there is an urgent need for a better understanding of population structuring for these species to aid management. Here we present 12 microsatellite loci developed for *S. melops* that also partly cross-amplify with *C. rupestris and L. bergylta* and are thus usable as a method for detecting potential population structure in these species.

We employed the company GIS (Genetic Identification Service Inc.) for the development of tetra repeat loci. The colony production and libraries were performed the following way: Recombinant plasmids included in the microfuge tubes were produced by ligating restriction fragments from *S. melops* DNA into the *Hin*dIII site of pUC19 plasmid. The fragments were enriched for a microsatellite motif. Ligation products were introduced into *E. coli* strain DH5" (ElectroMaxJ, Invitrogen) by electroporation. GIS used 2:1 of ligation mix for each of the libraries. Libraries were prepared from genomic DNA fragments being 350–700 bp long.

Sterilized toothpicks were used to transfer white colonies from the spread stock plates onto a bluo-gal/IPTG/ampicillin LB (BIA-LB) plate that has a transparent grid taped to the bottom (samples enclosed). This plate was

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Table 1 Primer sequences and characteristics of 12 corkwing wrasse (*Symphodus melops*) microsatellite loci

Locus	GenBank accession no.	T_a (°C)	Repeat motif	Primer sequences (5′–3′)	Size range (bp)	N_A	H_E	H_O	F_{IS}	P value
SmA11	xxx	56	$(GTTT)_9$	F: GACTCCATCCTGCTTCATC R: AGGCTGTAAAGAAATCAATCC	136–156	5	0.591	0.714	−0.213	0.259
AmA107	xxx	56	$(GTTT)_6$	F: TCAAGTCCTGAAGTTTTACTCA R: GCAGGTTGTATGTTTGAGG	139–159	5	0.544	0.677	−0.054	0.313
SmC8	xxx	56	$(TACA)_{22}(TTCA)_3$	F: TTTCCTGTATTAGATGGATGGA R: TACTCCCAATGGATCAAGTG	142–170	8	0.733	0.625	−0.250	0.091
SmD3	xxx	56	$(CTAT)_{10}$	F: GGGCTGCTAAATCTTGTTTG R: TCGTCCATCATAAAGTGGTG	286–342	10	0.765	0.818	−0.071	0.051
SmD110	xxx	56	$(TAGA)_{13}$	F: TAAACCATTTATGGACCTGGAC R: CTGCGTGCTCATCTTTAGTATG	262–290	6	0.651	0.710	−0.090	0.745
SmD112	xxx	56	$(CTAT)_{17}$	F: CCTCGGGATCAATAAAGTATC R: AGGGTAAACAGTGGACATTTAG	121–169	8	0.649	0.740	−0.144	0.589
SmD121	xxx	56	$(CTAT)_8$	F: TGCAACATTAAGGGTGAGC R: ATGGTAAGCTAGGCACTATGAG	245–339	12	0.752	0.777	−0.034	0.782
SmD128	xxx	56	*	F: GCCAGTTTAGGAGTATCGC R: CAAAGGCTTCTATCTGTCTGTC	235–295	16	0.938	0.521	0.449	0.0001***
SmA103	xxx	56	$(CAAA)_8$	F: TGAGCCAAGCAGTGAGTG R: GTGGGTGTCGTTTTCCAG	213–226	5	0.754	0.812	−0.078	0.072
SmB11	xxx	56	**	F: TCGCTAGAGGTAGCCTGACTG R: TAGGCACACAGAACGAAACAC	182–206	5	0.455	0.464	−0.078	0.587
SmD131	xxx	56	$(TAGA)_{10}$	F: CCTTTGTCTCCCTTCTGTG R: GCTTCATTTGCTGTCACTCT	147–163	5	0.488	0.531	−0.091	0.943
SmD134	xxx	56	$(TAGA)_{19}$	F: TGCAGGGATGAACATCTTACTC R: TGAACCATAAAGCATTAGCACA	144–224	18	0.919	0.969	0.055	0.632

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 32 individuals. Uncorrected P values for two-sided tests, * $P < 0.05$, *** $P < 0.001$

* $(TGGA)_7(GATA)_{37}(GACG)_8(GACA)_2$; ** $(TAGG)_3(TGGA)_4(TAGA)_{18}$

Table 2 PCR cross-amplification of all microsatellite loci in *Labrus bergylta* and *Ctenophora rupestris* developed for *Symphodus melops* (cf. Table 1)

Locus	Size range (bp)	N_A	H_E	H_O	F_{IS}
L. bergylta					
SMA103	na	na	na	na	na
SMD112	179	1	0	0	0
SMD121	na	na	na	na	na
SMD131	na	na	na	na	na
C. rupestris					
SMA103	198	1	0	0	0
SMD112	na	na	na	na	na
SMD121	235–237	2	0.400	0.25	0.391
SMD131	87–91	2	0.233	0.25	0.077

Four primers successfully amplified and were partly found to be polymorphic for both species ($n = 8$ per species). 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. As only eight individuals were used, the HW estimates is only indicative of possible deviations

incubated overnight, and colonies were selected from this plate rather than from the original spread. The procedure above largely follows Meredith and May (2002) and Schwartz and May (2004). 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 using DesignerPCR, version 1.03 (Research Genetics, Inc.). These primers were tested against library DNA plus DNA from seven individuals resulting in 12 polymorphic and reliably amplifying loci.

Population screening was conducted by analysing of 32 individuals, caught near the capital of Norway, Oslo (59.54 N; 10.44 E). 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. Only one locus, SMD128, deviated significantly from Hardy–Weinberg equilibrium in the GENEPOP probability tests. The locus was estimated to contain 27% null alleles (Chakraborty estimate) by MICROCHECKER. No other evidence for null alleles or Hardy–Weinberg deviations was found. No linkage disequilibrium (LD) was detected between pairs of loci (using GENEPOP). Finally, we cross amplified all loci with eight individuals of two related species *Ctenolabrus rupestris and Labrus bergylta*y resulting in four useful microsatellite DNA loci (Table 2). It is worth noting that the phylogenetic relationship between *S. melops* and the other two species is not very close (Hanel et al. 2002) and probably causing the primer loci to give a lower success in cross-amplification than anticipated between closely related species (see Knutsen et al. 2009).

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