Conservation Genet Resour (2014) 6:425–428
DOI 10.1007/s12686-013-0114-3

MICROSATELLITE LETTERS

Isolation and characterization of twenty microsatellite loci for the ballan wrasse, *Labrus bergylta*

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Received: 23 December 2013 / Accepted: 25 December 2013 / Published online: 3 January 2014

Abstract We developed 20 nuclear microsatellite DNA markers from tri- and tetra-nucleotide enriched libraries in the ballan wrasse. In our dataset (N = 241), the detected number of alleles per locus ranged from 2 to 12, and the observed and expected heterozygosity varied from 0.251 to 0.778 and from 0.286 to 0.804, respectively. Cross amplification with the goldsinny wrasse resulted in two usable loci whereas the corkwing wrasse amplified in one locus. The ballan wrasse is an important resource for aquaculture as it delouses farmed salmon efficiently and removes organic matter from farming facilities. In order to obtain individuals for this industry, the wrasse are translocated along the Norwegian coastline, in spite of no knowledge of the population structure. This paper enables such studies, which might be important for the long term management of the species.

Keywords *Labrus bergylta* · Ballan wrasse microsatellites · Salmon delouser

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The ballan wrasse, *Labrus bergylta* (Ascanius 1767) is the biggest of the labrids (Parenti and Randall 2000) in Scandinavian waters. This species is exploited commercially as biocontrol in the aquaculture industry, removing lice and organic matter from the sides of cages and nets, thus reducing the environmental impact of salmon farming. It is also popular as food fish and as catch-and-release target for sport fishermen.

Genomic DNA was extracted from fin tissue of 15 individuals using the Qiagen DNeasy Blood & Tissue Kit following manufacturer's instructions. Commercial company Genetic Identification Services (GIS Inc., Chatsworth, USA; http://genetic-id-services.com) was requested to develop and screen two multiplexed libraries enriched for four tetranucleotide (TAGA), (CATC), (AAAC), (CAGA) and four trinucleotide motifs (ATC), (AAC), (AGT), (ACC) following their proprietary protocol (Meredith and May 2002). A total of 368 clones were sequenced and 92 microsatellites were found. PCR primers for 76 microsatellite-containing sequences were designed using DesignerPCR v.1.03 (Research Genetics, Inc.). Some 43 primer pairs successfully amplified and 22 were polymorphic on a sample of eight individuals.

We assessed the variability of those polymorphic loci in 241 specimens collected from five sites in Norway. Six multiplexed PCR reactions were performed in final volume of 10 µl containing 50 ng DNA template, 1 × buffer, 2 mM $MgCl_2$, 1.25 mM dNTPs, 0.06–0.12 µM of each primer and 1U GoTaq polymerase (Table 1). PCR profiles included an initial 4 min denaturation at 94 °C followed by 24 cycles of 50 s at 94 °C, 90 s at an annealing temperature of 56 °C, 1 min of extension at 72 °C and a final extension of 72 °C for 10 min. Forward primers were labelled with fluorescent dyes and PCR products were electrophoresed on an ABI Prism 377 Genetic Analyzer

Table 1 Primer sequences and characteristics of twenty ballan wrasse, *L. bergylta* microsatellite markers tested on 241 individuals

Locus	GenBank accession no	Primer sequence (5′–3′)	Repeat motif	Multiplex	dNTP (μM)	*N*	*K*	Size	H_o	H_e	F_{IS}	PI
Wr-A103	KF971349	F: 6FAM-TGGTTGCTACCAAATCATG R: GGGACAGAATGAAATATCTCTG	$(GTT)_9$	1	0.12	172	6	191–200	0.698	0.650	−0.071	0.161
Wr-A107	KF971350	F: NED-GAAAGAGACGGACAGAGACA R: CGTCCCTATTTCATTGTCAC	$(AAC)_9$	1	0.06	241	3	188–197	0.303	0.314	0.037	0.499
Wr-A111	KF971351	F: VIC-ATCCAACAAATGGACTTAGTCA R: AAACGGAGACCAGTGGAG	$(TCTA)_{18}$	2	0.12	238	10	198–238	0.618	0.599	−0.028	0.203
Wr-A112	KF971352	F: 6FAM-CTCATGCGTGTTGAGATGT R: TTTATTTGCGCGACACTT	$(GTT)_9$	3	0.12	239	10	176–204	0.778	0.804	0.034	0.060
Wr-A113	KF971353	F: PET-TTGGAATCAAACAACCTCTC R: GAGCCTACAAATTATCATTGGT	$(GTT)_{17}$	1	0.12	240	7	198–228	0.742	0.747	0.0097	0.105
Wr-A203	KF971335	F: 6FAM-GATAGCGGGATAAAAGAAGATC R: TTCTATTTGGCAACCTTTACAC	$(GTT)_{14}$	2	0.12	239	12	168–219	0.724	0.716	−0.009	0.122
Wr-A223	KF971336	F: PET-CAGTGGGGAGATATAAGGATAA R: GTGGACCTGTCCTCTGTTAA	$(TAGA)_{10}$	5	0.12	238	3	277–293	0.332	0.306	−0.082	0.524
Wr-A224	KF971337	F: NED-GGACTGGGAACAGTTAAGATG R: CATGCGAGAGTTTTTCAAAG	$(ATC)_9$	2	0.1	238	4	174–195	0.500	0.509	0.020	0.360
Wr-A228	KF971338	F: VIC-AGGAAAACAGAGCCTACAAATT R: CTTGCTCCAGAACATTTCAG	$(AAC)_{12}$	1	0.12	239	7	167–196	0.736	0.747	0.016	0.105
Wr-A236	KF971339	F: 6FAM-TGAAATGTTGGTGGTGAAG R: CAACGCACTGAAGGTCAT	$(AAC)_9$	4	0.12	237	5	194–208	0.700	0.698	−0.001	0.138
Wr-A237	KF971340	F: PET-TTTCCCCCTTTAGCTTTGAG R: CAAAAACTACGTCTGCCATTTT	$(GTT)_9$	6	0.1	236	2	146–153	0.449	0.500	0.103	0.375
Wr-A254	KF971341	F: NED-CTTCTGTGACAGGGCTGATT R: GCCGCTGTTTGTGTGTAC	$(GTT)_{12}$	6	0.1	232	4	182–200	0.474	0.698	0.323	0.141
Wr-A255	KF971342	F: VIC-TGAAGGCAACAGGTCACG R: CCGACTCGTTATGGAGCAG	$(AAC)_9$	6	0.06	233	5	135–150	0.442	0.535	0.176	0.318
Wr-A256	KF971343	F: 6FAM-TGGCAAAGAAAAAGAACACAC R: AGGTGGAACATTGAACAAAAAC	$(TCTA)_{11}$	6	0.06	235	5	153–169	0.681	0.665	−0.021	0.182
Wr-A259	KF971344	F: PET-CTGCTGTGTCAACCCTAACT R: TGGCAGTTGTTAATGATGG	$(GTT)_{11}$	3	0.12	239	5	186–198	0.573	0.531	−0.076	0.289
Wr-A261	KF971345	F: NED-TCAAGAGGCGAGATGAGA R: GGAGAGAAATGAACCAATGA	$(ATC)_8$	3	0.12	238	5	223–237	0.408	0.391	−0.041	0.407
Wr-B102	KF971354	F: NED-GAAGGAATCTAGGGTTCAAATG R: CATGCCGAACAGTGTCAG	$(AAAC)_6$	4	0.1	240	2	200–208	0.288	0.286	−0.003	0.551
Wr-B212	KF971346	F: VIC-TCAGGTTGATACCGATTTCA R: GCAGTCAATAAAGGCTTTTACA	$(CAGA)_{11}$	5	0.12	238	7	167–199	0.588	0.608	0.035	0.194

Table 1 continued

Locus	GenBank accession no	Primer sequence (5′–3′)	Repeat motif	Multiplex	dNTP (μM)	*N*	*K*	Size	H_o	H_e	F_{IS}	PI
Wr-B213	KF971347	F: 6FAM-GATGGACAAAGTCAGGTGTG	$(AAAC)_6$	5	0.12	238	10	219–263	0.748	0.709	−0.053	0.134
		R: AGCGGTCAAGAGAAGTGG										
Wr-B215	KF971348	F: PET-GGAAACAAAGTGAGTAAGTGCA	$(TAA)_9$	4	0.12	239	3	170–185	0.251	0.323	0.224	0.508
		R: GATTTGGCAGCATTTCATATC										

The fluorescent dye (6FAM, PET, VIC or NED) is depicted in each case. N, number of individuals genotyped; k number of alleles, size indicates the range of observed alleles in base pairs; observed (H_o) and expected (H_e) heterozygosity; F_{IS}, inbreeding coefficient and PI, probability of identity for each locus

(Applied Biosystems). The 500LIZ size standard (Applied Biosystems) was used to accurately determine the size of the fragments and allelic variation. Conditions and characteristics of the loci are provided in Table 1.

Fragments were analyzed with the software GeneMapper v5 (Applied Biosystems), and Micro-Checker with 1,000 randomizations (Van Oosterhout et al. 2004) was used to evaluate presence of null alleles, stutter bands and large allele dropout. The number of alleles per locus (k), the observed and expected heterozygosity (H_o and H_e), the inbreeding coefficient (F_{IS}), the probability of identity (PI) and Hardy–Weinberg equilibrium tests (HWE) for each locus were calculated using GenAlEx 6.5 (Peakall and Smouse 2006). Linkage disequilibrium (LD) between pairs of loci was tested using GENEPOP on the web (Rousset 2008).

Twenty of the primers managed to amplify successfully in multiplex and produced an average of six alleles per locus. The duplicated genotyping of the 241 individuals revealed no inconsistencies in 12 of the loci whereas in the eight remaining ones, the percentage of mismatches ranged between 0.21 and 0.65 %. Micro-Checker suggested homozygote excess in loci Wr-A254, Wr-A255 and Wr-215. The averaged observed heterozygosity over all loci (H_o) and the level of expected heterozygosity (H_e) were 0.552 and 0.557 respectively. After FDR correction for multiple comparisons, three loci showed significant deviations from expectations under HWE and one case of no LD was detected among the 190 paired loci comparisons. Cross-species amplification was tested on the goldsinny wrasse, *Ctenolabrus rupestris,* which amplified at loci Wr-A237 and Wr-B102; and on the corkwing wrasse, S*ymphodus melops* that amplified at Wr-B102.

Acknowledgments Funding was provided by the Norwegian Ministry of Fisheries and Coastal Affairs. We are grateful to Anne Grete Sørvik Eide, Bjørghild Breistein Seliussen and Geir Dahle for their valuable assistance in the laboratory.

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