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TECHNICAL NOTE

Isolation and characterization of microsatellite markers in the southern hake, *Merluccius australis*

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Abstract Twenty-six nuclear-encoded microsatellites were isolated from an enriched genomic library of southern hake, *Merluccius australis*, and characterized in 30 individuals. The microsatellites include 18 dinucleotide repeats, seven trinucleotide repeats (two imperfect), and one pentanucleotide repeat. Southern hake support important industrial and artisanal fisheries in Chile, Argentina, and New Zealand.

Keywords Microsatellites · Southern hake · *Merluccius australis*

The southern hake, *Merluccius australis*, is a demersal, merlucciid species found primarily from 40°S in the Southeast Pacific (Chile) southwards around the southern tip of South America, to 38°S in the Southwest Atlantic (Argentina). A displaced, genetically distinct form of *M. australis* also occurs in waters off New Zealand south of 40°S (Machado-Schiaffino et al. 2009). Southern hake comprise one of the most important fisheries in the Southern Hemisphere (Machado-Schiaffino et al. 2009), including both industrial and artisanal fisheries along its distribution range (Horn 1997; Payá and Ehrhardt 2005). Catches (artisanal and industrial) of southern hake in Chilean waters, for example, averaged 30,000 t annually from 2000 to 2005 (Payá and Ehrhardt 2005), and in 2007 the export (industrial) value of southern hake was US $44.6 million and accounted for 41% of the total export volume and 57% of the total value of 'wild-caught' fish in Chilean waters (Globefish: Hake Market Report—June 2008).

Proper management of an exploited fishery requires stock-structure assessments, both within and across political boundaries. Nuclear-encoded microsatellite markers are well suited for population-genetic analysis and identification of stocks over both smaller geographic ranges, such as the spawning and nursery habitats in Chilean fjords (Bustos et al. 2007), as well as across the multiple political boundaries spanned by *M. australis* (Ward 2000). Here, we report on the development of polymerase chain reaction (PCR) primers for 26 microsatellites from an enriched *M. australis* genomic DNA library.

Details for the generation of the enriched microsatellite library can be found in Renshaw et al. (2010). Two separate hybridization reactions were performed. One reaction was with 50 pmol of 3′-biotin-modified $(CA)_{13}$ oligonucleotides, while the other was with 50 pmol of 3′-biotin-modified $(CAA)_8$ oligonucleotides. Hybridization mixtures were heated to 95°C for 10 min and then kept at 55°C [$(CA)_{13}$ hybridization] or 49°C [$(CAA)_8$ hybridization] for 1.25 h. Enriched genomic fragments were ligated into the pCR®2.1-TOPO® vector (Invitrogen) and transformed into *Escherichia coli* (One Shot® TOP10 Chemically Competent Cells, Invitrogen). Positive (white) clones were sent to the Interdisciplinary Center for Biotechnology Research at the University of Florida (http://www.biotech.ufl.edu/) for sequencing with M13 primers. Resulting sequences were edited and vectors trimmed with SEQUENCHER 4.1 (Gene Codes). Primer pairs were developed using PRIMER3 (http://frodo.wi.mit.edu/).

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Table 1 Summary data for 26 microsatellites characterized for the southern hake, *Merluccius australis*

Msat	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone size[d]	N/N_A[e]	Size range[f]	H_E/H_O[g]	P_{HW}^h
*Mau*6	CATCAGGTAGGGCATCCAGT[FA] CCCAGTAGGGCACCTTGTTA	HM641695	$(CA)_8$	272	29/7	292–308	0.601/0.690	0.114
*Mau*8	GTTTGCTGGTAGCAGTGCAT[FA] GGGAAGAAGGGGAAAGTGAG	HM641697	$(CT)_{12}$	323	26/12	334–402	0.790/0.808	0.475
*Mau*9	GCGGTCTCCTGCTAATTGTT[FA] CGCCTGTCTTGTCATTCTCA	HM641696	$(CA)_7$	119	29/4	132–138	0.477/0.448	0.158
*Mau*11	CCAGGGAATATCGACCCTTTA[FA] CGTGTCATTTCCCAACAAAA	HM641698	$(CA)_{12}$	283	29/4	304–310	0.666/0.655	0.005
*Mau*14	TGCCTAGGTGCTGACTGATG[NE] GGGGTCAAACTGTCTGAGGT	HM641699	$(GT)_{10}$	190	20/9	200–224	0.833/0.850	0.570
*Mau*15	CGAGAGACACCGAGGAAGAG[FA] GCCACACACATTACCCGTCT	HM641700	$(GA)_{16}$	134	26/17	140–192	0.918/0.885	0.725
*Mau*16	TTAGCGCGAGAGATGGAAAT[FA] AACTCACACGGGCATACACA	HM641701	$(GT)_{11}$	167	28/13	182–214	0.842/0.821	0.401
*Mau*18	CCTCTCTACGTGGCAGAACC[FA] GTCCGTTATCCCCCACTCAT	HM641702	$(GA)_{18}$	139	30/24	141–209	0.952/0.933	0.476
*Mau*23	GTCCGAGATGCATCCCTTTA[FA] AACCCCAGCTGTGTGTGTG	HM641703	$(CA)_{17}$	289	29/5	299–315	0.255/0.207	0.092
*Mau*24	TCTGTGTCACACTCACTCACTCA[FA] AGACCCTCGGGTTAATGGTT	HM641704	$(CA)_{14}$	252	26/9	255–277	0.514/0.577	0.968
*Mau*25	GGCACCCATAGACGAGTGAT[NE] TTTGACATGGCTTTGCACAC	HM641705	$(GT)_{11}$	235	30/5	259–267	0.218/0.233	1.000
*Mau*28	GCAGAGTGCCACACGTACAC[NE] GCGTCGGTATGTGGTGAGTT	HM641706	$(CA)_7$	149	30/5	167–175	0.497/0.433	0.638
*Mau*31	CGCACATTAATGATTAGCCTGA[FA] TCAACATCCCACATGTGTCC	HM641707	$(CAA)_8$-6 bp-$(CAA)_8$	273	29/10	265–319	0.840/0.655	<u>0.002</u>
*Mau*33	CGAGGATTACCGAGGAGATG[FA] GTCAACAGTGGGGAGGTTGT	HM641708	$(AC)_7$	80	30/2	99–101	0.155/0.167	1.000
*Mau*34	TCAGGAACCCTGGTCAATTC[FA] TCCCTGCAGGAGAGAAGAGA	HM641709	$(CTT)_{28}$	219	27/18	220–304	0.927/0.889	0.028
*Mau*35	GGAGTAGCTTCGGACCATTC[FA] GCGACATTAGAGGGCAAAAA	HM641710	$(ACT)_{12}$	300	29/8	318–348	0.704/0.828	0.346
*Mau*36	ATGGGCAGGGTTTAGTCCTT[NE] CGCTAAGTGGATCACAAGCA	HM641711	$(AC)_7$	152	30/3	171–175	0.508/0.567	0.086
*Mau*37	TGGCACATCACACACCTACA[NE] GTCCCCTTGCATTTGACACT	HM641712	$(GTT)_{14}$	223	30/15	238–280	0.923/0.867	0.500
*Mau*39	ACACACTGGATGCACACCAT[FA] AAAGCGTTTATGTGCCCAAC	HM641714	$(CA)_{10}$	146	30/8	156–182	0.685/0.700	0.890
*Mau*41	ATTGAATTCCGTCCTCAACG[FA] CACTCTAGAGGCAGATCTGTGG	HM641715	$(AAC)_8$	140	30/4	151–163	0.324/0.267	0.090
*Mau*44	CATGTTGTATGCCGAGGTTG[FA] AGAGCCTCTTCCACACGAAG	HM641716	$(CA)_{47}$	179	30/33	152–240	0.975/0.933	0.074
*Mau*45	CCTGGTAACTACATTTGTTGGTTTT[FA] AGGTGCTTCATGTTGCTGTG	HM641717	$(CA)_{14}$	207	28/24	219–285	0.957/0.929	0.121
*Mau*49	GAAGGTCAATGGGGGAAAAA[NE] CCCTACCTGCCGTACAAGAA	HM641718	$(CAA)_4$	145	29/2	163–166	0.034/0.034	1.000
*Mau*50	GCTTACCATTTACTGGTAGAGA[FA] CCCTTTATGTTGTCATATCCAT	HM641719	$(TAG)_4$-3 bp-$(TAG)_4$	95	29/3	102–117	0.164/0.172	1.000

Table 1 continued

Msat	Primer sequence (5′–3′)[a]	GenBank[b]	Repeat[c]	Clone size[d]	N/N_A[e]	Size range[f]	H_E/H_O[g]	P_{HW}^{h}
*Mau*51	GCTGAACGCCTGGTTTACTC[FA] CGCAGAAAGACTTCGACACA	HM641720	$(TTTCA)_4$	305	29/5	321–341	0.284/0.241	0.278
*Mau*53	CGCACACTGTCTCCCTCTCT[NE] GAGGGAATGGGAAATGGAGT	HM641721	$(CA)_5$	150	28/2	169–171	0.070/0.071	1.000

[a] Primer sequences are forward (top) and reverse (bottom)

[b] GenBank Accession number

[c] Repeat indicates repeat motif

[d] Clone size is the size (in base pairs) of the allele in the sequenced clone

[e] N is the number of individuals assayed, and N_A is the number of alleles detected

[f] Size range refers to alleles thus far uncovered (includes the 21 bp 5′-tail-sequence)

[g] H_E and H_O are expected and observed heterozygosity, respectively

[h] P_{HW} represents the probability of deviation from Hardy–Weinberg expectations (significant deviations are underlined). The fluorescent 5′-tail-sequence label attached to the forward (top) primer was either 6-FAM[FA] or NED[NE]

PCR amplifications included three primers. Unlabelled forward and reverse primers were purchased from Integrated DNA Technologies (IDT); the forward primer included an extra 21 bp tail-sequence (5′-GCCTCGTTTA TCAGATGTGGA-3′). The third primer, the 21 bp 5′-tail-sequence labeled with either 6-FAM or NED fluorescent label (Set D, Applied Biosystems), enabled amplicons to be fluorescently labeled (see Boutin-Ganache et al. 2001 for more details). Each primer pair was initially evaluated with a subset of 13 individuals and successful amplifications were further evaluated with an additional 17 individuals (30 individuals total). All samples came from waters off the Chilean coast. PCR products were run on an ABI 377 automated sequencer. Alleles were sized using the GENESCAN®-400 HD ROX Size Standard (Applied Biosystems); allele sizing and calling were performed using GENESCAN® version 3.1.2 and GENOTYPER® version 2.5 software (Applied Biosystems). Genetic variability of the microsatellite markers was measured by number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity as calculated in GDA (Lewis and Zaykin 2001). A Fisher's exact test, as implemented in GDA (Lewis and Zaykin 2001), was used to test for significant departures from the expectations of Hardy–Weinberg equilibrium at each microsatellite and for significant departure from genotypic equilibrium at each pair of microsatellites. Evidence for occurrences of null alleles was explored using MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004).

Of the initial 55 putative microsatellites identified, 26 PCR primer pairs produced experimentally tractable microsatellites. Summary data for these 26 microsatellites are presented in Table 1. The number of alleles detected ranged from two (*Mau*33, *Mau*49, *Mau*53) to 33 (*Mau*44); expected heterozygosity ranged from 0.034 (*Mau*49) to 0.975 (*Mau*44), while observed heterozygosity ranged from 0.034 (*Mau*49) to 0.933 (*Mau*18, *Mau*44); genotypes at one microsatellite (*Mau*31) deviated significantly from Hardy–Weinberg expectations following Bonferroni correction (Rice 1989). Analysis using MICROCHECKER indicated no evidence for scoring error due to stuttering or large allele dropout at any of the 26 microsatellites; the possible presence of null alleles was suggested at *Mau*31. None of the pair-wise comparisons of microsatellites deviated significantly from genotypic equilibrium following Bonferroni corrections (Rice 1989). The microsatellites characterized in this study will aid in selective breeding programs and prove useful for future population-genetic studies of *M. australis* as well as other members of genus *Merluccius*.

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