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MICROSATELLITE LETTERS

Isolation and characterization of polymorphic microsatellite loci from the chinstrap penguin, *Pygoscelis antarctica*

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Abstract Sixteen polymorphic microsatellite loci were isolated and characterized for the chinstrap penguin, *Pygoscelis antarctica*. The number of alleles per locus ranged from two to nine. The observed and expected heterozygosities ranged from 0.136 to 0.864 and from 0.208 to 0.831, respectively. Cross-species amplification was successful in other penguin species, including *Pygoscelis papua, Pygoscelis adelie,* and *Eudyptes chrysolophus*. The microsatellite markers developed in this study can be used in future conservation studies.

Keywords *Pygoscelis antarctica* · Pygoscelid · Chinstrap penguin · Microsatellites

The chinstrap penguin, *Pygoscelis antarctica,* is one of three pygoscelid penguin species predominant in the Antarctic Peninsula and surrounding islands. Although global population sizes have increased, some island or peninsular populations directly influenced by reduced sea ice cover have undergone a sudden decrease (Korczak-Abshire et al. 2012). We isolated and characterized 16 polymorphic microsatellite loci in *P. antarctica* using the Illumina HiSeq 2000 platform (Illumina, San Diago, CA, USA) for use in future conservation studies. Blood samples from seven individuals were collected from Narębski Point (an Antarctic Specially Protected Area, 62°14′S, 58°48′W) on the Barton Peninsula on King George Island in the austral summer 2009–2010. Blood samples were taken from the wing vein using a syringe and stored in EDTA tubes. Total DNA was extracted from the blood samples using the DNeasy blood and tissue kit (Qiagen, Venlo, Netherlands). Isolation of microsatellite regions was conducted using QDD3 software (Meglécz et al.2014). Thirty microsatellite regions showing possible variation between individual sequences were selected for use in the following procedures. Variability of the loci was screened by genotyping 23 individuals from the same locality. The polymerase chain reaction (PCR) primers for the amplifications were designed using Primer 3 software (Rozen and Skaletsky 2000). The forward primers included M13 tails attached at the 5′ end (FAM: TTTCCCAGTCACGACGTTG, VIC: TAAAACGACGGCCAGTGC and PET: GCGGATAACAATTTCACACAGG), and the reverse primers were pigtailed at the 5′ end (GTTTCTT). A multiplex PCR was performed in a reaction mixture (final volume of 16 μL) containing 8 μL of 2× Multiplex PCR master mix (Qiagen), 50 ng of DNA template, 0.05 μM of each M13-tailed forward primer, 0.5 μM of each pigtailed reverse primer, and 0.1 μM of each fluorescently-labeled M13 primer. The PCR cycle conditions were as follows: initial denaturation at 95 °C for 15 min, 41 cycles of denaturation at 95 °C for 30 s, annealing at a specific temperature for 90 s (seven cycles at 65 °C, seven cycles at 61 °C, seven cycles at 58 °C and 21 cycles at 55 °C), elongation at 72 °C for 30 s, and a final extension at 72 °C for 20 min. The PCR products were genotyped using a 3130xl Genetic Analyzer

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Table 1 Characterization of 16 microsatellite loci from *Pygoscelis antarctica*

Locus	Primer sequences (5′–3′)	Repeat	N_A	Size range (bp)	H_O	H_E	GenBank accession number
Multiplex set 1							
Pygantarc02	FAM-CTGGCGGAGCTTCTTACTTG CTGACATTATGTTGAGCCTTGC	$(AT)^9$	2	111–113	0.455	0.397	KP316211
Pygantarc11	VIC-GGGGGAGTGCATATCAGAAA CATCAAAGAATTCGCCTTGG	$(AT)^{10}$	4	192–198	0.455*	0.567	KP316215
Pygantarc24	PET-TGAACAAAGGGAAAGGCAAC CTGGGGCAAGGAATACTGAC	$(AC)^9$	3	322–326	0.591	0.582	KP316221
Multiplex set 2							
Pygantarc03	FAM-TCTTTGTGCTTTGAGTCCCA GCTACTGCAGAGCAATTCACA	$(AC)^8$	3	114–118	0.409	0.478	KP316212
Pygantarc16	VIC-TTTGAGATGCTGGGAAAAAGA CAGACTGCATGGAGAAGCAA	$(AC)^{18}$	9	208–226	0.727	0.826	KP316217
Pygantarc22	PET-TGTGAGGGTGCTTGTGTGAT GGGAGTTTTTCTCCCAGTCC	$(AC)^{11}$	2	325–330	0.136	0.268	KP316220
Multiplex set 3							
Pygantarc19	VIC-GTATTCCCATGCAGGCTTTG CAAGCGTGTTTTTGTTTGCT	$(ACAGAT)^5$	4	206–236	0.409	0.447	KP316219
Pygantarc26	PET-ATTGCTGTGGGTCATTTGTG CAAGTCTCCAACTCAATGAACA	$(AT)^6$	3	333–338	0.227	0.208	KP316223
Multiplex set 4							
Pygantarc06	FAM-ACCTGACGATAGCTGGCCTA TTCAACAGGGAAACAGAAGTGT	$(AC)^5$	2	119–121	0.409	0.325	KP316213
Pygantarc15	VIC-AAGATCTGCCTCCTGCATTC CAATGGCTGATTCCCCAATA	$(AAT)^{14}$	8	232–253	0.909	0.831	KP316216
Pygantarc28	PET-TCCTCTTTGGAGAGGCAACT AGGTGGGAAAAGCAGCATC	$(AC)^8$	3	392–413	0.682	0.515	KP316225
Multiplex set 5							
Pygantarc07	FAM-CCCATCAGAGTGACGTTCAA TATTTGACAGCCTTCCTGCC	$(AAGG)^6$	2	125–133	0.545	0.496	KP316214
Pygantarc18	VIC-TCGGCTGTTTCACTTTTTCA CAAGCAATCCATTTGCACTT	$(AGAT)^{12}$	5	256–272	0.864*	0.702	KP316218
Pygantarc25	PET-TCAGTGAATAACTGGCAAGCA TGTGCTAGTTTGCGACCAGA	$(AG)^8$	3	413–427	0.455	0.555	KP316222
Multiplex set 6							
Pygantarc27	PET-TGGGAGACTGTGCTTCCATT TTCAGTGGGTCTTCCAAATGT	$(AC)^9$	4	386–391	0.500	0.507	KP316224
Multiplex set 7							
Pygantarc29	PET-TGTCCAAAATCCTGGAAAGTG CCAAAAGAGCCCTGAGCA	$(AG)^{10}$	3	308–316	0.591	0.616	KP316226
Mean			3.75		0.523	0.520	

N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

* Significant departure from Hardy–Weinberg equilibrium ($p < 0.05$)

(Applied Biosystems, Carlsbad, CA, USA) and the sizes were scored using GeneMapper v4.0 Software (Applied Biosystems). The number of alleles (N_A) and observed (H_o) and expected heterozygosities (H_E) were estimated using GenAlEx 6.5 (Peakall and Smouse 2012) and Hardy–Weinberg equilibrium tests were performed using Genepop 4.2 (Rousset 2008). From the 30 primer sets designed, 29 microsatellite loci were successfully amplified, and 16

Table 2 Results of cross-species amplification with primer pairs developed from *Pygoscelis antarctica*

Loci	Species		
	Pygoscelis papua	*Pygoscelis adelie*	*Eudyptes chrysolophus*
Pygantarc02	+	+	+
Pygantarc03	+	+	−
Pygantarc06	+	+	+
Pygantarc07	+	+	+
Pygantarc11	+	+	−
Pygantarc15	+	+	−
Pygantarc16	+	+	+
Pygantarc18	+	+	−
Pygantarc19	+	+	+
Pygantarc22	+	+	+
Pygantarc24	+	+	+
Pygantarc25	+	+	+
Pygantarc26	+	+	+
Pygantarc27	−	−	−
Pygantarc28	+	+	+
Pygantarc29	−	+	+

Two individuals of each species were screened

+ amplification, − no amplification

primer sets were found to be polymorphic. The number of alleles per locus ranged from two to nine (Table 1). The observed heterozygosities ranged from 0.136 to 0.864, and the expected heterozygosities ranged from 0.208 to 0.831. Two loci (Pygantarc11 and Pygantarc18) showed significant deviations from Hardy–Weinberg equilibrium. Cross-species amplifications conducted for three other penguin species, *Pygoscelis papua, Pygoscelis adelie,* and *Eudyptes chrysolophus*, showed successful amplification of the loci (Table 2).

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