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TECHNICAL NOTE

Microsatellite loci for genetic analysis of the arctic gadids *Boreogadus saida* and *Arctogadus glacialis*

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Abstract We report sets of 19 and 16 microsatellite loci for the examination of the population genetics of *Boreogadus saida* and *Arctogadus glacialis*, respectively. Six of these loci were developed from a collection of 9,497 expressed sequences from *B. saida* while the remaining loci were found in the literature and optimized for use in *B. saida* and *A. glacialis*. The numbers of alleles observed for each locus ranged from 3 to 33 in *B. saida* and 1–22 in *A. glacialis*. Observed heterozygosities ranged from 0.02 to 0.93 in *B. saida* and 0.17–1.0 in *A. glacialis*. Species specific differences were observed for the loci providing new tools for the identification of these two morphologically similar arctic gadids. The loci presented here can be used to distinguish between the two species and fill fundamental biological knowledge gaps, thus promoting conservation of these important fishes.

Keywords *Boreogadus saida* · *Arctogadus glacialis* · Arctic cod · Polar cod · Population genetics · Species identification · Human impacts · Climate change

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The arctic gadids *Boreogadus saida* and *Arctogadus glacialis* are small, mainly planktivorous fishes distributed throughout the Arctic Ocean. These gadids are a staple food for many taxa (Bradstreet et al. 1986; Loseto et al. 2009). Increasing global temperatures and human activities in the Arctic will have impacts on the habitat and water properties that are critical to these important fishes (Barber et al. 2008; Lasserre 2010). An incomplete understanding of the biology, habitat use and population structure of these fishes make the prediction of their response to climatic and anthropogenic driven change difficult. Molecular genetics is an effective way to fill some of these critical knowledge gaps.

Compared to other species of similar ecological importance, there is far less known about the genetics of *B. saida* and *A. glacialis*. The DNA sequence of the entire mitochondrial genome of both species has recently been determined (Breines et al. 2008). An early study using random amplified polymorphic DNA markers (RAPD DNA) on *B. saida* from the North Atlantic did not detect differentiation at the population level (Fevolden et al. 1999). More recently, analysis of the mitochondrial DNA (mtDNA) of *B. saida* from waters around Greenland detected two mtDNA lineages but no strong population differentiation (Pálsson et al. 2009). Another factor in the examination of the population genetics of *B. saida* and *A. glacialis* is the difficulty in discrimination between these

two species based on their morphological features. A genetic species identification test (Madsen et al. 2009) allows for the discrimination of both species but necessitates further refinement to be applied across the entire range of these fishes. In sum, there is clearly a need for highly variable nuclear markers such as microsatellite DNA loci to allow for further examination of population differentiation and species identification of *B. saida* and *A. glacialis*. Here we report the development of microsatellite DNA loci that will be useful for such studies.

Total RNA from a whole single specimen of *B. saida* was extracted in TRIzol reagent (Invitrogen, Carlsbad, CA) by mixer-mill homogenization (Retsch, Newtown, PA, USA) and spin-column purified using RNeasy Mini kits (Qiagen, Valencia, CA, USA). A normalized expressed sequence tag (EST) library of the RNA was directionally constructed in the pAL-17.3 vector by Evrogen, Moscow, Russia. The library was plated and robotically arrayed in 384-well plates. Plasmid DNAs were extracted and BigDye™ Terminator (Applied Biosystems, Foster City, CA, USA) cycle

Table 1 Information regarding repeat sequence, Genbank accession number (Acc. no.), primer sequence (including fluorescent tag), PCR annealing temperature (T_a), and reference, for microsatellite loci useful for *B. saida* and *A. glacialis*

Locus	Repeat	Acc. no.	Primer 1	Primer 2	T_a	References
Bsa6	CA	HO070596	FAM-CTC TAG AGC GTT TTG TCT CC	AAC CAT TTG TTT TGG TAC AGG	52	This study
Bsa7	CA	HO070778	FAM-TCT TGG AGA AAA GGA ATC GG	AAA AGG TAC ACG ACA AAC CG	52	This study
Bsa14	GATA	HO071740	HEX-CGA TAC TAT AGC TGC AAA CGC	ATG AAA TGC TAT CCG ACT CC	52	This study
Bsa15	GATT	HO071607	HEX-CTC CTT CAT CTG TGG TCA GC	GAA GAC ACC TCG TCA CGC	52	This study
Bsa60	TGAA	HO077536	HEX-AAA GGG TTC ATT CAA AAG GG	GCT TTC ATC TCA AAA CAC CC	52	This study
Bsa101	GATA	HO078113	FAM-TGT TAA TGC TGC TTC TTT GC	GTG CTT GTG TGT GTT TCA GC	52	This study
Gmo8	GACA	AF159238	FAM-TGG GGG AGG CAT CTG TCA TTC A	GCA AAA CGA GAT GCA CAG ACA CC	52	Miller et al. (2000)
Gmo32	TTG	DQ191392	HEX-CAA TCG CCG TCC AAC CAA C	GGC GGC AGC AAC GAT TCT C	52	Jakobsdottir et al. (2006)
Gmo34	GACA	AF159234	FAM-TCC ACA GAA GGT CTC CTA A	GGT TGG ACC TCA TGG TGA A	52	Miller et al. (2000)
Gmo127	CAGA	EU735055	HEX-TCT GGT GCA GAT CCT CGA TG	TCA GAG GTT CCG GTC GTA AG	58	Skirnisdottir et al. (2008)
GmoC18	ACA	CO542701	HEX-AAG CAT GCG TTT GTG TTA TTAC	ATC TGT TCT CGC TTT CCT TCA TT	52	Stenvik et al. (2006)
GmoC20	CAT	CO542424	HEX-CTG CCA AAG CCT GTG ACG	GAT GGT GGT GTT GAT TGT GGT TGT	52	Stenvik et al. (2006)
GmoC71	CA	CO541968	HEX-TGA CGA TAC ATT CAA GAG CAC CAC	AAG AAC CAG CAC ACG ATT TGA CA	52	Stenvik et al. (2006)
GmoC86	CT	CO542534	5Cy3-CAAGTC CCC AAA TGA AAC CTC	GCC CTC GCT GTC GTC GTA ATA AG	52	Stenvik et al. (2006)
GmoC102	TCA	n/a	5Cy3-TCA ACC GTA TTC CTT TCG CAT TAT	AAG CCG CCC GTC GTT CAG AGT A	52	Delghandi et al. (2008)
GmoC122	AC	n/a	5Cy3-AGG GGA TTG CTG GGT GTC ATT	AAG CAT TGT GGA GCC ATT TGT ATC	52	Delghandi et al. (2008)
GmoC291	AT	EU860236	FAM-CAA GTC CCC AAA TGA AAC CTC	GTT TCT TTT ATA AAT CCT TGG TTC TCG TGT T	52	Delghandi et al. (2009)
Tch5	GATA	AF178495	FAM-TCG CAT TGA GCC TAG TTT	GCC TTA ATA TCA CGC ACA	52	O'Reilly et al. (2000)
Tch14	GAAA	AF178504	FAM-CAT ACA TTG GTC ACT CTT TCT TAC	AAA CTG ATA TAC GCC CAA CT	52	O'Reilly et al. (2000)

n/a not available

sequenced using an ABI 3730 sequencer using conventional procedures and the following primers: M13 forward (5′-GTAAAACGACGGCCAGT-3′), and SP6WAN (Evrogen, Moscow, Russia). The resulting ESTs were assembled with CAP3 (Huang and Madan 1999) with default parameters and placed into GENBANK accession numbers HO069577 to HO079074. Primers for polymerase chain reaction (PCR) were designed from 150 microsatellite bearing-ESTs using PRIMER3 (Rozen and Skaletsky 2000). These primers were tested on crude DNA extracts prepared from six *B. saida* specimens according to methods in Nelson et al. (1998). Polymerase chain reactions were performed in a final volume of 10 µl in GoTaq Flexi Buffer (Promega, Madison, WI) containing 1.5 mM MgCl2, 0.08 mM each nucleotide, 0.25 U of Taq DNA polymerase, and 0.4 µl crude DNA extracts diluted 1/15 in 10 mM Tris 1 mM EDTA pH 7.4. Primer pairs that showed promise for population genetics studies of *B. saida* and *A. glacialis* from this effort, and from screening previously reported primers as above, are shown in Table 1. The following cycling parameters were used in the PCR: 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, T_a for 30 s, 72 °C for 1 min, then 72 °C for 7 min. The size (in base pairs) of PCR products were determined with an Applied Biosystems 3,730 sequencer with ROX1000 size standard (Applied Biosystems, Foster City, CA). Alleles were scored using GENEMAPPER software (Applied Biosystems, Foster City, CA). To characterize allele size range in both species, specimens from multiple localities were examined. Population level statistics were calculated for a single locale sample of *B. saida* and a multi-locale sample of *A. glacialis*. The PCR annealing temperatures used along with characteristics of the PCR products are shown in Table 2. Calculation of concordance with Hardy–Weinberg equilibrium (HWE) was done with the program GENEPOP (Raymond and Rousett 1995) and calculation of observed heterozygosity (H_o) and expected heterozygosity (H_e) was done with GENETIX (Belkhir et al. 1996).

We identified 19 and 16 polymorphic microsatellite loci that can be used to examine population genetics of *B. saida* and *A. glacialis*, respectively, and that can also help in species identification. The information gained from future studies employing these loci will illuminate many of the outstanding basic questions in the biology of these two arctic gadids and will be of direct benefit for the design of

Table 2 Details of microsatellite loci amplification in *B. saida* and *A. glacialis*

Locus	*B. saida*							*A. glacialis*						
	N	AMP	A	SR (bp)	Ho	He	P[a]	N	AMP	A	SR (bp)	H_o	H_e	P[b]
Bsa6	237	*****	15	183–215	0.63	0.68	0.57	36	*****	13	165–191	0.85	0.83	0.59
Bsa7	237	*****	5	184–192	0.29	0.29	0.71	36	*****	14	174–196	0.75	0.84	0.16
Bsa14	237	*****	12	179–215	0.63	0.6	0.95	36	*****	13	203–227	0.68	0.76	0.04
Bsa15	237	*****	6	178–202	0.56	0.51	0.13	36	*	2	194–195	n/a	n/a	n/a
Bsa60	237	*****	11	189–229	0.73	0.73	0.77	36	*****	15	165–221	0.86	0.86	0.63
Bsa101	237	*****	10	131–159	0.71	0.76	0.60	36	*****	13	127–174	0.86	0.86	0.04
Gmo8	237	*****	5	128–202	0.1	0.1	0.14	36	*****	22	137–284	0.77	0.76	0.00
Gmo32	237	*****	3	101–105	0.1	0.2	<0.01	36	*****	5	98–106	0.18	0.17	0.04
Gmo34	237	*****	7	67–88	0.64	0.66	0.84	36	*****	4	75–83	0.17	0.57	0.00
Gmo127	237	*****	8	273–304	0.69	0.68	0.54	36	***	6	186–308	0.75	0.67	0.71
GmoC18	237	*****	25	129–193	0.86	0.88	0.06	20	*****	1	142	n/a	n/a	n/a
GmoC20	237	*****	11	115–147	0.52	0.62	<0.01	20	*****	6	115–131	0.60	0.58	1.00
GmoC71	237	*****	8	166–215	0.08	0.08	1.00	20	*****	2	196–198	0.47	0.46	1.00
GmoC86	237	*****	5	96–118	0.023	0.02	1.00	20	*	2	105–118	n/a	n/a	n/a
GmoC102	237	*****	17	77–125	0.88	0.88	0.40	20	*****	6	92–113	0.63	0.58	0.07
GmoC122	237	*****	6	153–165	0.075	0.07	1.00	20	*****	7	153–165	0.63	0.56	0.48
GmoC291	237	*****	5	150–162	0.02	0.02	1.00	20	*****	1	154	n/a	n/a	n/a
Tch5	237	****	33	206–326	0.87	0.95	0.16	20	0	0	n/a	n/a	n/a	n/a
Tch14	237	*****	28	110–216	0.93	0.94	0.73	20	*****	14	110–174	1.00	0.90	1.00

Amplification success: ***** 80–100 %; **** 60–79 % *** 40–59 %; ** 20–39 %; * 1–19 %; 0, no successful amplification seen

N numbers of individuals scored, *AMP* amplification success, *SR* observed allele size range, *A* observed number of alleles, and test of concordance with Hardy–Weinberg equilibrium

[a] He, Ho and conformation to HWE calculated for single location sample only ($N = 179$)

[b] Sample consists of a mixture of multiple different populations

n/a indicates either insufficient alleles for calculation, or insufficientsuccessful amplifications

regulations aimed at managing both short and long term impacts of industrial activities.

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