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TECHNICAL NOTE

Isolation and characterization of nuclear microsatellite loci in the northern shrimp, *Pandalus borealis*

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Abstract We developed and characterized 20 microsatellite primer loci for the northern shrimp *Pandalus borealis*. All 20 loci were polymorphic with number of alleles ranging from 3 to 36 and with observed heterozygosity between 0.04 and 0.93. In addition, we tested the utility of these markers in three related shrimp species, *P. montagui*, *Atlantopandalus propinqvus* and *Dichelopandalus bonnieri*. These new markers will prove useful in the identification of stock structure and hence, assessment of the commercially important species *P. borealis*.

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Evidence is accumulating that many marine species are spatially structured into reproductively isolated populations to certain extent. The recognition and understanding of the mechanisms responsible for such population structure is of paramount importance for fishery management and species conservation (Ruzzante et al. 2006; Schindler et al. 2010).

The northern shrimp *Pandalus borealis* is an abundant benthic species in the North Atlantic, being commercially important in its whole distribution. Previous genetic studies have rendered information of its genetic structure in the North Atlantic. Kartavtsev et al. (1991) showed no genetic differences at four allozyme loci among three localities in the Barents Sea. Rasmussen et al. (1993) also used allozymes to show significant differences between Svalbard and two northern Norwegian fjords, but showed genetic homogeneity within all the compared regions. Jónsdóttir et al. (1998) compared the Denmark Strait with inshore and offshore localities of northern Iceland and found significant allozyme differences among all three areas. Drengstig et al. (2000) found genetic divergence between Norwegian fjords, but widely separated localities in the Barent Sea showed no differentiation. The use of allozymes has proven useful to resolve some genetic structure among ocean-wide samples (Grant et al. 1998); however, large differences at particular loci may question their neutrality for population genetic studies. Another study using RAPD analysis (Martinez et al. 2006) showed *P. borealis* from the Norwegian fjords and Jan Mayen stations different from the Barents Sea and Svalbard. The two localities at Jan Mayen also differed from each other but no further intraregional

Table 1 Primer sequences and polymorphism statistics for 20 microsatellite loci in two populations of *Pandalus borealis*

Locus name	Primer sequence (5′–3′)	Repeat motif in library	Multiplex	T_a (°C)	Size range (bp)	Oslofjord			Sola		
						n	*N*a	H_O/H_E	*n*	*N*a	H_O/H_E
SD1–30	F: TGGATATCTACGCCCATTATGT R: TACGTCCCAGCAGAGGCTAT	$(CA)_6CTA(CA)_{24}$	1	60	340–348	48	8	0.596/0.727*	38	7	0.571/0.616
SD1–41	F: TGATAGAAGAGAGAAGATGAAGGAAA R: GCGTCTTTGGCTTACGAATC	$(GTA)_5(GTAGTT)_3(GTA)_{17}$ ATA$(GTA)_3$	1	65–61	315–351	48	11	0.915/0.866	38	13	0.829/0.854
SD3–62	F: TTGCCACAGACACAGAAACG R: AGAACGTGCAGGCATCCTAT	$(GT)_6$	1	60	131–141	48	3	0.255/0.311	38	3	0.189/0.176
SD1–18	F: TGGATATCTACGCCCATTATGT R: CCTTACCCTACGTTCCAGCA	$(AC)_{25}$	2	56	348–354	48	7	0.596/0.715	38	8	0.541/0.711*
SD2–14	F: CAAATTTGATGCGATTCACG R: GACGTTATCTTGCACGCAGT	$(AC)_7(GC)_3\ldots(AC)_8$	2	55	136–158	48	10	0.660/0.748	38	10	0.711/0.714
SD2–35	F: TGGCTAGAAGACAGGGCAAT R: CGGCCCTTTATTCCATCATT	$(CA)_{17}$	2	54	204–298	48	28	0.915/0.922	38	25	0.811/0.917*
SD2–68	F: TGATGGTGAACACAGGTACG R: TGGTGAATTCAGTTACCGATCA	$(AC)_{10}GC(AC)_7$	2	54–50	166–172	48	4	0.638/0.608	38	10	0.737/0.687
PbB115	F: GCTGCTCATTTTTGCCTATC R: CGATGGATGGATGAATAGATG	$(CATC)_{17}CATT(CATC)_2$	3	50.5	126–142	48	4	0.250/0.409*	38	4	0.243/0.405*
PbB118	F: ATAGCGACACACATAAATACCA R: TTTGAGCCACAAGACATCA	$(TCCA)_{13}TCTATCCA(TCCA)_3$	3	51.8	152–188	48	7	0.581/0.750*	38	6	0.605/0.727*
PbC109	F: TGGGATAGCCAATACGAAG R: CCTTAGGAAACTGGAATCATC	$(AC)_8(ATAC)_{11}(AC)_3AT(AC)_{19}$	3	50.5	101–207	48	29	0.917/0.895	38	22	0.838/0.935
PbA1	F: GGGCAGGTTATTAGGAAGG R: TTGTGAGCAGGTCCAAGAC	$(GTTT)_9$	4	52.4	162–206	48	9	0.688/0.770	38	11	0.703/0.792
PbA103	F: TCTTCCCACCGAGTGTAAT R: GATGGCTTGGATTGGATT	$(CAAA)CACA(CAAA)_4$	4	49.9	129–153	48	6	0.500/0.761*	38	7	0.649/0.766
PbA104a	F: ATCACGCAAACGCACATAA R: TCGGGAAGAACTGAACAGG	$(CA)_2(CAAA)_6TAAA(CA)_7$	4	53.0	184–200	48	3	0.250/0.341	38	2	0.316/0.337
PbC106	F: CCCAGAAATCCTGGTTACG R: CGTAGGAAATACAGTGCTGATG	$(GACA)_2(CA)_9(TACA)_{20}$ $(GACA)_{25}$	4	52.9	116–380	48	36	0.617/0.926*	38	29	0.763/0.847
PbD8	F: TGTCTCTACATCTGCCTGTCTA R: CCTGATGATACGCTATTTGAG	$(CTAT)_{25}(CTGT)_{30}$	5	50.6	194–330	48	19	0.630/0.885*	38	19	0.771/0.923*
PbD9	F: TTCCATATAGCACACGTTGTC R: AACCAGACGAGAAGGTAGATTT	$(TATC)_3TAACTATGTATGTAAC$ $(TATC)_9$	5	52.6	231–291	48	9	0.681/0.788	38	8	0.703/0.816
PbA110	F: GGATGAGTCACCTGTGTATCTT R: GTCGGTATCTCAAGGCTCTTA	(GT)4$(GTTT)_6$	5	53.5	232–284	48	5	0.575/0.588	38	8	0.684/0.637

Table 1 continued

Locus name	Primer sequence (5′–3′)	Repeat motif in library	Multiplex	T_a (°C)	Size range (bp)	Oslofjord			Sola		
						n	*Na*	H_O/H_E	*n*	*Na*	H_O/H_E
PbC8	F: ATCGGTTTGGCTGTTATTGAG	$(TATG)_4TATA(TATG)_6$	6	53.2	276–352	48	14	0.773/0.734	38	10	0.526/0.594
	R: AGGATTGTTGATGAGGTGGAC	CACGTAAC $(TATG)_{11}$									
PbA108	F: CATTGCTGACAGTGTTTCTCC	$(GTTA)_5(GTTT)_6$	6	53.7	248–268	48	3	0.147/0.237	38	2	0.039/0.111
	R: TAGCCCATTTCTGACCTATCC										
PbC105	F: ATGATTCAAAATCTGGTGTCAC	$(ATAC)_{22}…(GA)_{28}$	6	51.3	172–300	48	12	0.933/0.848	38	10	0.790/0.831
	R: CTTACGTTTCAACTTTGTCACC										

Repeat motif is listed 5′–3′ with respect to the forward primer (F)

T_a, annealing temperature; *n*, number of samples analyzed; *Na*, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity (i.e. gene diversity; Nei 1987). Asterisk denotes significant deviations from HW expectations ($p < 0.01$)

differences were reported. RAPD markers may be valuable to diagnose traits or for genetic mapping studies but their dominance and both their reproducibility and homology issues raise concerns about their suitability for population genetic studies. Here, 20 microsatellite loci for the shrimp *P. borealis* are reported that will be useful in future studies of population genetic structure in this species.

Two different approaches were chosen for the isolation of the microsatellite loci. The first approach was performed at the Department of Marine Ecology—Tjärnö, University of Gothenburg. Here, a microsatellite-enriched genomic library was developed from *P. borealis* following a modified protocol from Glenn and Schable (2005). Briefly, DNA was simultaneously digested with *Rsa*I and ligated to SNX linkers, then hybridized to $(AC)_{15}$ and, $[(GATA)_7$, $(ATCT)_7$ and $(TGTA)_7]$ biotinylated oligoprobes and enriched DNA recovered by polymerase chain reaction (PCR). PCR products were ligated into a TOPO® Vector and transformed following the manufacturer's protocol (Invitrogen). Recombinant colonies were screened by PCR in 25 µl reactions, using one primer for the vector (M13 [–20]) and a second containing the oligonucleotide repeat mix: $(AC)_{15}$ or $[(GATA)_7$, $(ATCT)_7$ $(TGTA)_7]$. A total of 192 positive clones were sequenced and primer pairs were designed for 33 microsatellite arrays of suitable size (5–20 repeats) using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). A subset of 12 PCR primer pairs was tested on eight shrimps for tractability (reproducibility, consistency, range in allele size, presence/frequency of "stutter" bands, and polymorphisms).

The second approach was through the commercial company Genetic Identification Services (GIS Inc., Chatsworth, USA; http://genetic-id-services.com) to develop and screen four libraries enriched for tetranucleotide (AAAC), (CATC), (TACA), and (TAGA) motifs, following their proprietary protocol (Meredith and May 2002; Schwartz and May 2004). A total of 96 clones were sequenced and 16 primer pairs were designed.

Thus, a total of 28 primer pairs were included in the initial test. Subsequently 21 of these were further tested on a larger number of samples. The microsatellite loci were arranged in six multiplexes (Table 1). The PCR were done in 2.5 µl reaction volumes with 1× Qiagen multiplex kit, 0.1–0.5 µM primers and 15–25 ng DNA. The primers (Applied Biosystems, Foster City, CA) were labeled with fluorescent dye at the 5′ end of the forward primer (Table 1). The amplifications were performed in a GeneAmp PCR system 9700 (Applied Biosystems) with the following PCR profile: An initial denaturation step at 95°C for 15 min, followed by 35 cycles of 95°C for 30 s, 56°C for 3 min and 72°C for 60 s, ending with a final elongation step at 60°C for 30 min. Fragment sizes were determined with an ABI 3130 XL automated sequencer (Applied

Table 2 Cross-species amplification of microsatellite loci from *Pandalus borealis*

Locus	*Atlantopandalus propinqvus* ($n = 13$)			*Pandalus montagui* ($n = 14$)			*Dichelopandalus bonnieri* ($n = 12$)		
	Size range (bp)	Na	H_O/H_E	Size range (bp)	Na	H_O/H_E	Size range (bp)	Na	H_O/H_E
PbA104a	176–238	15	0.846/0.938	172–234	19	0.857/0.966	–	–	–
PbC109	265–329	10	0.308/0.895*	–	–	–	223–245	8	0.917/0.895
PbA1 1	117–191	11	0.385/0.911*	–	–	–	117–123	4	0.250/0.750*
SD1–41	129–285	3	0.154/0.151	–	–	–	216–330	4	0.250/0.652*
SD2–68	153–167	5	0.462/0.769*	153–161	4	0.357/0.669	155–165	6	0.583/0.844

Na, H_O/H_E are according to Table 1

* Indicates deviation from HW ($p < 0.01$)

Biosystems) and analyzed using Genemapper 4.0 (Applied Biosystems).

Allele frequencies, observed and expected heterozygosities, Hardy–Weinberg proportions and linkage disequilibrium were calculated using ARLEQUIN 3.5 (Excoffier and Lischer 2010).

In total, 48 individuals from Oslofjord and 38 from Sola, Norway were used to characterize the selected loci. The 20 polymorphic microsatellite loci presented here showed from 3 to 36 alleles with observed heterozygosities ranging from 0.04 to 0.93 (Table 1, Genbank no. GF111281–GF111300). The significant deviations from Hardy–Weinberg expectations were all associated with heterozygote deficiency. One locus pair combination showed significant deviation from linkage equilibrium (SD1–18 and SD1–30 in Oslofjord).

Cross-amplification was tested in 12–14 individuals of three other shrimp species: *P. montagui*, *Atlantopandalus propinqvus* and *Dichelopandalus bonnieri*. Five loci were polymorphic in *A. propinqvus*, 4 in *D. bonnieri* and 2 in *P. montagui* (Table 2).

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