Wordcount = 1000 words

Isolation and characterization of 12 microsatellite loci in the Peruvian scallop *Argopecten purpuratus* and cross-species amplification in other scallop species (family Pectinidae)

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Abstract *Argopecten purpuratus* is an economically important marine bivalve. Twelve microsatellite loci were isolated and characterized in 20 individuals of *A. purpuratus* collected in Isla Independencia (Pisco, Peru) and tested for cross-species amplification in other 4 scallops (family Pectinidae). Ten loci were polymorphic. The number of alleles per locus ranged from 2 to 13. The observed (H_O) and expected (H_E) heterozygosities ranged from 0.100 to 0.900 and from 0.185 to 0.903, respectively. Two loci showed significant deviation from Hardy-Weinberg equilibrium. A low rate of cross-species amplification was found.

Keywords *Argopecten purpuratus • Pectinidae • Microsatellites • Genetic diversity*

The Peruvian scallop, *Argopecten purpuratus*, is an economically important species widely exploited in Peru and Chile. In spite of its great commercial and ecological value, little is known about its genetic variation and population structure and to date, there is only one article on the development of microsatellite loci in this species (Pickerell *et al*. 2004). Thus, further studies using more microsatellite markers are required to obtain a more detailed population genetic insight. Here we report the successful development and characterization of 12 new microsatellite markers and their cross-species amplification in other four economically important scallop species from the family Pectinidae.

Genomic DNA was isolated following the standard phenol-chloroform protocol from one individual of *A. purpuratus*. Microsatellite enrichment library was constructed using magnetic beads hybridization selection protocol. Approximately 5 µg DNA was digested with *Hae*III (Roche), after partial digestion, DNA was size selected (500–1500 bp) with Qiaquick gel extraction kit (Qiagen). Subsequently, recovered DNA was dephosphorylated and ligated to SNX linker (Hamilton *et al*. 1999) and then amplified by PCR using SNX forward primer. For the enrichment library, two consecutive hybridizations were performed. Purified linker ligated DNA was hybridized to biotinylated $(CAT)_8$, $(GAT)_8$ and $(GATA)_4$ probes (hybridization temperatures were about 5 degrees below Tm of each probe). Hybridized DNA was captured with streptavidin-coated magnetic beads (Dynal) and subjected to stringency washes to remove unhybridized DNA. Recovered DNA was PCR amplified with SNX forward primer. Amplified fragments were ligated to pCR4-Topo vector and transformed into TOP10 One Shot chemical competent *Escherichia coli* cells (Invitrogen). Colonies were screened by PCR using M13(-20)-F and M13-R primers, a total of 376 positive clones were detected and sequenced using the BigDye terminator v3.1 Cycle Sequencing Kit with M13(-20) forward primer on an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems). A total of 26 microsatellite containing sequences were selected for primer design, and 2 forward and 2 reverse primers were designed for each potential locus depending on their flanking sequences length. All microsatellite loci were PCR screened in a total of 20 *A. purpuratus* individuals collected from Isla Independencia, Pisco, Peru. After PCR standardization and visualization in agarose gels, 15 primer pairs were selected for further screening by fragment analysis sequence. PCR reactions were carried out

as described by Morishima *et al*. (2008). Briefly, 100 ng of template DNA was amplified in a final volume of 10 μL mix reaction containing 40 μM dNTPs, 0.3 pmol M13-tailed forward primer, 3.0 pmol reverse primer, 3.0 pmol fluorescence labeled M13 primer and 0.025 U *Taq* polymerase (TaKaRa, Otsu, Japan), and thermocycling conditions were as follow: initial denaturation for 3 min at 95˚C, followed by 35 cycles of denaturation for 30 s at 95˚C, annealing for 30 s at 56-60˚C (Table 1), and extension for 30 s at 72˚C, followed by a final extension for 45 min at 72˚C. PCR products were sequenced using formamide and GeneScan LIZ-500 size standard (Applied Biosystems). We used GeneMapper 3.7 software (Applied Biosystems) for allele scoring and GENEPOP version 4.0.10 (Raymond and Rousset 1995) was used to test for departures of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium and ARLEQUIN 3.5 (Excoffier and Lischer 2010) to calculate the observed (H_O) and expected (H_E) heterozygosity.

Of the 15 loci tested, 12 generated consistent results. Ten of the 12 primer pairs were polymorphic and the number of alleles per locus ranged from 2 to 13. The observed (H_O) and expected (H_E) heterozygosities ranged from 0.100 to 0.900 and from 0.185 to 0.903, respectively (Table 1). Two pairs of loci were in linkage disequilibrium (APPE11-APPE17 and APPE13-APPE22) after Bonferroni correction and only two loci (APPE20 and APPE26) showed significant deviation from HWE, probably due to the presence of null alleles. Previous studies have reported that null alleles are common in bivalve mollusks (Hedgecock *et al*. 2004; Wang and Guo 2007; Wang *et al*. 2010) and are known to cause deviation from HWE and to underestimate heterozygotes in natural populations (Wang *et al*. 2010).

Cross-species amplification was performed for all the newly developed microsatellite in four other related scallops (Table 2): *A. irradians* (Qingdao, China), *Pecten maximus* (Rye bay, England), *P. albicans* (Shimoda, Japan), and *Mizuhopecten yessoensis* (Hakodate, Japan); however, only five microsatellites loci were successfully amplified for *A. irradians* and four of them were polymorphic (APPE11, APPE17, APPE18 and APPE23); in *P. maximus*, the loci APPE23 and APPE25.1 amplified with a single allele, for *M. yessoensis* only the loci APPE23 and APPE26 could amplify and both showed monomorphism. No successful amplification was detected in *P. albicans* samples. Poor microsatellite

transferability among related species has also been observed in previous studies in marine bivalves (Zhan *et al*. 2005; Hedgecock *et al*. 2004).

The polymorphic loci described herein will be useful to estimate gene flow, genetic diversity and to study the population structure of *A. purpuratus*.

Table 1 Characterization of 12 microsatellite loci in *A. purpuratus*

Locus	Primers (5'→3')	Repeat motif	T_a (˚C)	Size (bp)	N_a	n	H_O	H_E	DDBJ access number
APPE9	F: GATCTCAGTAAGACCTATGGCA R: GCTTTTGCTTATTTGGGTTG	$(ATC)_{18}…(AAC)_7$	56	254-296	13	18	0.900	0.903	AB649042
APPE11	F: TTGATCTGCGTGTCTTCCGT R: CGTCAGTGGTTGTTTCTGGTC	$(GAT)_8...(GAT)_5$	56	177-239	10	15	0.750	0.863	AB649043
APPE13	F: ACACTTAACCATGTCCTATGC R: GCATCATCCTCTTATGGCA	$(GAT)_6…(GAT)_8$	56	180-313	9	20	0.700	0.747	AB649044
APPE17	F: GAGACTAAAGGAAGCATTGT R: CCGCAGTCAAACTAACTTAAAC	$(CAT)_{26}$	56	369-455	11	20	0.900	0.855	AB649045
APPE18	F: TCGGTCGAAGCCGAAGTAGAGC R: TGTCATCAGCAACTTCATCTAC	$(GAT)_2(GTT)_3..(GAT)_2(GTT)_3...(GAT)_2(GTT)_3$	56	380-547	4	19	0.600	0.624	AB649046
APPE20	F: ATACAACGACGGCGGCAGCAA R: TGGGCCAGCACTTGTAAAAGCC	$(GAT)_7…(GAT)_5…(GAT)_7…(GAT)_8$ $(GGT)_7...(GAT)_9$	56	327-563	8	19	0.100*	0.854	AB649047
APPE22	F: GACCTTGAACTTTGACAC R: GCCTAAGTTCTGTAGCACCATAG	$(GAT)_8$	56	273-357	7	15	0.700	0.738	AB649048
APPE23	F: TCTGTCATGAGTCATTGATGG R: AGTTCCAGTTCAGGATCCAC	$(GAT)_7$	60	177-183	2	20	0.200	0.185	AB649049
APPE25.1	F: GCTACCACCAGTGTTTCCAT R: TACAACCAGCAGGGATTCGGT	$(CCA)_3(CCG)_6$	60	148-160	4	20	0.550	0.568	AB649050
APPE26	F: CCAGAGTACGCCCCGAAAATAC R: ATGCCGTGTATGTAACAGGGAAA	$(CAGA)_{17}(GATA)_7(GACA)_2$	60	221-225	2	20	0.900*	0.508	AB649051
APPE3	F: GATATACGCATGCCGTGTATGTAA R: TTCCAACAACCTCAAAACAGTCC	$(ATCT)_3CTC(AT)_4…(ATCT)_3TT(CT)_4$ $(GTTT)_2$	56	289	1	16			AB649040
APPE6	F: GCCATCCCTGGACTTATTAATAGTA R: CCTGTAGAGAATTCCTGTGAAGA	$(CT)_{14}$	56	207	1	16			AB649041

Primer sequence, repeat motif, optimized annealing temperature (T_a), allele size (bp), allele number (N_a), number of individuals genotyped (n), expected (H_E) and observed (H_O) heterozygosity and DDBJ access number are given for each locus. *Indicates significant deviation from HWE after Bonferroni correction.

Table 2 Cross-species amplification results of 12 *A. purpuratus* microsatellite loci in 4 related scallops: *A. irradians*, *P. maximus*, *P. albicans* and *M. yessoensis*

Locus	*A. irradians*				*P. maximus*				*P. albicans*				*M. yessoensis*			
	n	N_a	Size range bp	T°	n	N_a	Size range bp	T°	n	N_a	Size range bp	T°	n	N_a	Size range bp	T°
APPE9			X				X				O				X	
APPE11	16	7	153-158	56			O				O				O	
APPE13			X				X				X				X	
APPE17	16	5	327-381	50			X				X				X	
APPE18	14	3	379-461	56			O				O				X	
APPE20			X				O				O				O	
APPE22			X				O				X				X	
APPE23	16	2	173-187	60	2	1	179	56			X		10	1	174	53
APPE25.1	16	1	141	50	2	1	149	53			O				O	
APPE26			O				X				X		10	1	315	50
APPE3			O				X				X				X	
APPE6			X				X				X				X	

n: number of individuals screened; N_a: number of alleles; size range (bp); T°: annealing temperature; X: no amplification and O: multiple bands pattern or product of unexpected size.

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Acknowledgements We thank Ruben Alfaro and Elmer Ramos for providing *A. purpuratus* samples, Linsheng Song for *A. irradians* samples and Takuma Ozaki and Aya Yamada for their helpful advice.