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Thirty-Seven Additional Microsatellite Loci in the Pacific Lion-Paw Scallop (*Nodipecten subnodosus*) and Cross-Amplification in Other Pectinids

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TECHNICAL NOTE

Thirty-seven additional microsatellite loci in the Pacific lion-paw scallop (*Nodipecten subnodosus*) and cross-amplification in other pectinids

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Abstract We characterized 37 new microsatellite markers in the Pacific lion-paw scallop (*Nodipecten subnodosus*) and tested for cross-amplification in four other species. Genetic diversity was estimated using 24 individuals from the Lagoon Ojo de Liebre, B.C.S., Mexico. Allelic richness varied from 5 to 27 alleles per locus and the average expected heterozygosity was 0.76. Ten loci exhibited significant departure from Hardy–Weinberg equilibrium likely due to the presence of null alleles. Sixteen of these markers cross-amplified in closely related *N. nodosus*, while little or no amplification was observed in three *Argopecten* species.

Keywords *Argopecten* · Ojo de Liebre · Oligos · STR

Prior work identified 35 polymorphic microsatellite loci in the Pacific lion-paw scallop (*Nodipecten subnodosus*) (Ibarra et al. 2006) for use in conservation and population genetics of this large pectinid. Aggregations of this species are found around the coast of the Baja California Peninsula, Mexico, where they are both fished and cultured for human consumption. Like many marine species, populations have recently declined due to harvest pressures. In addition to characterizing the genetic diversity within and between populations, the development of microsatellite markers will allow for genetic mapping of the genome and eventually the discovery of the genetic basis of traits such as fitness and growth. With 19 chromosomes (N), and only 35 markers identified prior to this work, these additional markers are necessary to enable genetic mapping of this species and will also be valuable for continued population level investigations in the wild and in aquaculture.

Clones ($n = 480$) enriched for the repeats $(TAGA)_n$, $(TGAC)_n$, $(TACA)_n$, and $(ATC)_n$ from the mixed scallop-crayfish library described in Ibarra et al. (2006) were sequenced. These sequences were aligned with previously identified *N. subnodosus* microsatellites using Sequencher v4.7 (Gene Codes) to avoid duplication. Novel sequences were input into mReps (Kolpakov et al. 2003) to determine repeat regions and primers flanking the repeats were designed with Primer 3 (Rozen and Skaletsky 2000).

Because the enriched library was created from two species, the primers were first screened for amplification using genomic DNA of four *N. subnodosus* and four Shasta crayfish *(Pacifastacus fortis)* individuals. PCR reactions for the initial screening were identical to those in Ibarra et al. (2006). Thermalcycler conditions consisted of 3 min at 94°C followed by 31 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, and a final extension of 10 min at 72°C. PCR products were separated and visualized as described in (Ibarra et al. 2006).

Of the 106 designed primer pairs, 37 (34.9%) amplified and were polymorphic in *N. subnodosus*. For population screening and cross-amplification tests, the forward primer of each pair was either labeled with a 5′ fluorophore, or one of four 5′ modifications were added (universal primers: T7T, T7P, M13, SP6) using the method of (Schuelke 2000). In this method, the "tail" consisting of the universal primer sequence is incorporated into the template during early rounds of PCR. An additional third labeled oligo then

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Table 1 Characterization of 37 microsatellite loci in the Pacific lion-paw scallop (*Nodipecten subnodosus*) from the Lagoon Ojo de Liebre, B.C.S., Mexico. Shown are locus names, GenBank accession number, repeat motif, primer sequences, label system and PCR profile used in screening, number scored (N), number of alleles observed (A), allele size range, expected and observed heterozygosities (H_e and H_o, respectively), and *P*-value for tests of deviation from Hardy–Weinberg Equilibrium (HWE_{pv})

Locus & GenBank Accession	Repeat Motif[a]	Forward Primer (5′-3′) Reverse Primer (5′-3′)	Primer Label[b], PCR Profile[c]	N	A (Range)	H_e	H_o	HWE_{pv}
Nsub2A1A06	$(ATAG)_5AGG(TAGA)_7TA$	TTTCCTTTCGCCAAAACATC	IV	24	10	0.87	0.96	0.753
FJ986332		GCCTCTGAACCAAACACCAT			(164–220)			
Nsub2A1B12	$(ATCT)_9ATC$	TCCCGCGTAACTGAGAGACT	IV	19	12	0.82	0.47	0.008
FJ986334		AAGCTTGCACGATAAAGCGTA			(170–268)			
Nsub2A1C06	$(TCTA)_3TCT…C_{18}…(TC)_7(AC)_7…(CA)_9$	CTATTTGTGGTTGCGGGTGT	I	6	5	0.79	0.67	0.018
FJ986333		TGTGGTTAACGGTGGAATGA			(369–376)[d]			
Nsub2A1D12	$(TATC)_{10}(CA)_{15(}TACA)_6T$	GACAAACAAAAGGCGCTGAT	III	21	25	0.97	0.67	0.000*
FJ986335		ACACGTGGGTTTGAAGGAAG			(476–550)			
Nsub2A1F01	$(ATCT)_7AC(CTAT)_6…(CTGC)_4CTG(TCTG)_3$ $(CCTG)_4CCT$	CGCACAGAGTCATTGACCAC	IV	22	23	0.96	1.00	0.483
FJ986336		AAGGCTTTTCAATGCTTCCA			(233–320)			
Nsub2A1H03	$(TCAT)_8TCA$	TGAGGCCGGATCGTAATAAG	IV	17	5	0.70	0.18	0.000*
FJ986337		TTTTGCATCGATTTGACAGC			(369–413)			
NsubA1A01	$(GAT)_9$	CGGCCAGTGTAACGGTAGAT	II	24	13	0.88	0.58	0.002*
FJ986338		CATCTCTCTCCATGCCCTGT			(254–313)[d]			
NsubA1B04	$(ATGG)_3ATG…(GAT)_6(TGA)_6T$	AGCAGAGGAAGCAAGTCTCTT	III	22	20	0.92	1.00	0.707
FJ986339		TTGCATACGAATGGGCTACT			(217–292)			
NsubA1B09	$(CAT)_{14}$	CTAGGACGGAAGGAAGATGG	II	24	15	0.93	0.96	0.947
FJ986340		TGTGTATTTCTTATCACAAGGTGAA			(193-232)[d]			
NsubA1B12	$(TCC)_4T(N)_6(CAT)_7C(GTC)_5GT$	GCTGGCATACGTGTTTTTCA	III	24	24	0.96	1.00	0.313
FJ986341		CCTCGTAGCACATGGTTGAA			(233–320)			
NsubA1C02	$(TCA)_8TC$	TTCCATAGTTTGTCTTATGTTTTCA	III	22	18	0.94	0.68	0.001*
FJ986342		AAAAGAGGGAAAACCCAATG			(171–209)			
NsubA1C07	$(ATC)_{13}A(ACA)_3AC$	CTATAACCCCTCGCACCATC	I	22	21	0.95	0.68	0.000*
FJ986343		AGCCTCGGGCTATCTCTCTC			(287–376)[d]			
NsubA1C08	$(TGA)_{12}T$	AGGCGAAATATCGAGTCCTG	II	24	11	0.86	0.67	0.076
FJ986344		CATCTCTCTCCATGCCCTGT			(359–402)[d]			
NsubA1C12	$(TGA)_8TG$	ACTGCACCAACAACAATGGA	I	20	6	0.76	0.80	0.667
FJ986345		CAGTTGCATCCTCCTCCTTC			(472–493)[d]			
NsubA1D10	$(TGA)_8T$	CCTCCAGGCTCATGTTCACT	III	21	8	0.85	0.48	0.000*
FJ986346		AAACGGAACAATCCGCTAGA			(144–166)			
NsubA1F03	$(ATG)_9AT$	ACAATGTGGCAATGATGACG	IV	23	11	0.69	0.57	0.083
FJ986347		TCATCCATAAGCATCCACCA			(148–185)			

Table 1 continued

Locus & GenBank Accession	Repeat Motif[a]	Forward Primer (5′-3′) Reverse Primer (5′-3′)	Primer Label[b], PCR Profile[c]	N	A (Range)	H_e	H_o	HWE_{pv}
NsubA1F04	$(TGA)_{10}(N)_6(TGA)_4$	AAGACCGCGCACTCTATCAT	I	24	18	0.92	0.79	0.036
FJ986348		AAATCATCGCGGTTGTCTTC			(277–363)[d]			
NsubA1F07	$(GAT)_7(N)_6(GAT)_5G$	TGTGTAATGAAATACCATTGACGAT	II	24	18	0.93	0.83	0.116
FJ986349		CACCCTCACCATCAAAATCA			(172–264)[d]			
NsubA1F12	$(GAT)_{11}GA(N)_5(GAT)_8G$	GATGCCAAAACATCGCAAG	II	24	17	0.91	0.92	0.858
FJ986350		GCTTCTTTAATCATCATTTCTTATTGG			(203–287)[d]			
NsubA1G09	$(CAT)_9C$	GCTATCTGCTGTGTGTGGACAA	IV	24	14	0.91	0.92	0.867
FJ986351		TGGGGAAATCCTTCCATGT			(342–372)			
NsubA1H09	$(ATC)_{20}A$	AAGGAAGACAATTTTATGACTCGTG	IV	24	13	0.87	0.92	0.186
FJ986352		AATGGTTGCAAGTGCCAGAT			(386–454)			
NsubA1H12	$(ATG)_{10}$	GGTTATGGTGTTGCTTCCTGA	IV	24	14	0.90	0.88	0.822
FJ986353		CCGTTCACTGACCGAGAGAT			(483–532)			
NsubA2C01	$(TGA)_{13}(CGA)_{10}CG$	AGAGGTTTGGTAAGGGCAGAT	IV	22	21	0.94	0.91	0.476
FJ986354		GGAATGTTTGTTTCAGGCTCA			(249–363)			
NsubA2D10	$(TCA)_4T$	TTCACTCAGACACACGCACA	I	21	5	0.56	0.57	0.284
FJ986355		CCGATGATATGATGGCTGCT			(201–273)[d]			
NsubA2F04	$(CGT)_4TT(TCA)_{21}TC$	ATTGGTGTCGTTGTCATCGT	I	24	17	0.91	0.92	0.561
FJ986356		GCTTCTTAATGCGTGTGACG			(136–186)[d]			
NsubA2F08	$(TGA)_7$	CGATGACGCTACCAGGAAGT	IV	22	10	0.86	0.50	0.001*
FJ986357		GCAAGCTTCTTAGCGTGGAG			(322–346)			
NsubA2H05	$(ATG)_4ATA(ATG)_7A$	GCTAATGCGACAGGCTAAGG	III	24	9	0.83	0.88	0.925
FJ986358		GTGCGTGCATATGTGATTCC			(394–431)			
NsubB1A02	$(CATA)_7C(ACAT)_{17}$	CATCCCTCCATCCAATCAGT	IV	19	14	0.88	0.79	0.031
FJ986359		GTGATCTCCCAGAGCAGGAG			(211–268)			
NsubB1C05	$(GT)_{12}GAGG(GTAT)_{10}$	CCTGTGATCAGCCACTTCAA	I	20	19	0.95	0.95	0.576
FJ986360		GCACCGAGTATTCCGATTTC			(377–462)[d]			
NsubB1C07	$(TATG)_4C(ATGT)_{15}ATG(ATGT)_{13}$ ATG	ACAGGTCCTGACGTTTCTGC	II	24	27	0.96	0.50	0.000*
FJ986361		CCTGGATTGGCAAGTCAAAT			(188–299)[d]			
NsubB1E11	$(ATAC)_{13}…(ATAC)_4A(N)_7(CGTG)_5C$	TACGTACGCCCACCACTACA	II	21	22	0.95	1.00	0.547
FJ986362		TGGCACCATGTAAGACAGACA			(304–499)[d]			
NsubB1F02	$(ACCT)_4AC(ATAC)_6A(CATA)_{15}CA$ $(ACAT)_3ACA$	GCTATTTTTGGTGCGTTGTG	I	21	16	0.91	0.52	0.000*
FJ986363		AAGAGGAATGTGCCTGCTGT			(313–443)[d]			
NsubB1G03	$(GTAT)_6A(TATG)_{14}T$	TTGCATGCACTGTAATTTTCG	IV	19	17	0.94	0.89	0.361
FJ986364		TATGCCCAGCGTCAATATCA			(289–410)			

Table 1 continued

Locus & GenBank Accession	Repeat Motif[a]	Forward Primer (5′-3′) Reverse Primer (5′-3′)	Primer Label[b], PCR Profile[c]	N	A (Range)	H_e	H_o	HWE_{pv}
NsubB207	$(ACAT)_7ACA\ldots(CT)_5C$	CGAAAATACGCAATCGGAGT	III	24	13	0.50	0.46	0.095
FJ986365		GGACAACAGGTGTCATGTCTG			(375–459)			
NsubB2B05	$(ACAT)_5ACA(ATAC)_{11}$	TGATGACCTGCGCAGTAAAG	I	21	15	0.90	0.90	0.180
FJ986366		TCGGCTAGACCAACGACTCT			(157–249)[d]			
NsubB2C04	$(CATA)9\ldots(ATAC)_{15}AATG\ (CATA)_{16}$	TGGTGTCATGACCTGGATTG	I	21	22	0.94	0.81	0.002*
FJ986367		ATGTCTCGCTACTGGCAGGT			(210–412)[d]			
NsubB2C05	$(CTCA)_6CT$	TGTACATGTATTATCGCTATCAACTCG	III	24	19	0.92	0.79	0.081
FJ986368		ACGGCGGTGTCAAATAAATC			(267–334)			

* Significant after sequential Bonferroni correction

[a] Spaces in repeat motif indicate a stretch of non-repetitive genomic sequence

[b] Forward primers of markers with profiles I and II were tailed while those with profiles III and IV were 5′ end-labeled with either 6-FAM, VIC, NED, or PET

[c] (I) 9 cycles at 68 (TD -2°/cycle) followed by 21 cycles at 50°C; (II) 28 cycles at 56°C followed by 10 cycles at 53°C; (III) 31 cycles, 56°C; (IV) 31 cycles, 52°C

[d] Size range includes the M13, SP6, T7T, or T7P tail

targets these tails, incorporating the fluorophore and allowing for visualization of the fragment. The four tails were each labeled with a different fluorophore (T7T = PET, T7P = NED, SP6 = VIC, M13 = 6-FAM) allowing for multiplexing of PCR reactions.

Twenty-four *N. subnodosus* collected from the Lagoon Ojo de Liebre on the Pacific coast of the Baja California Peninsula, Mexico, were genotyped at all 37 markers. Amplification was attempted for three individuals each of *Argopecten irradians* (USA, west Atlantic), *Argopecten purpuratus* (Chile, east Pacific), *Argopecten ventricosus* (Mexico, east Pacific), and *Nodipecten nodosus* (Venezuela, west Atlantic) to assess cross-amplification success.

For primers directly labeled with 5′- 6-FAM, VIC, NED, or PET, 10 µl PCR reactions included 5–10 ng of genomic DNA, 1X buffer (Roche), 2 mM $MgCl_2$, 0.2 mM of each dNTP (Promega), 10 pmol each primer, and 0.4U FastStart Taq DNA polymerase (Roche). The thermal cycler profile was identical to the initial screen, except annealing temperatures varied depending upon the marker (Table 1).

PCR reactions for tailed primers differed by using 7.5 pmol tailed forward primer, 22.5 pmol reverse primer, and 1.5 pmol of fluorescently labeled oligo. The thermal cycler profile for tailed primers consisted of 5 min at 94°C followed by a marker dependent number of cycles of 94°C for 45 s, T_a for 90 s, and 72°C for 1 min, additional cycles of 94°C for 45 s, T_a for 90 s, and 72°C for 1 min, and a 10 min final extension at 72°C (see Table 1 for T_a and cycle number).

PCR products were diluted with 70 µl nanopure water and 1.5 µl was added to 8.8 µl of highly deionized formamide (Gel Company) and 0.2 µl of LIZ600 size standard (ABI). The samples were denatured for 3 min at 95°C before electrophoresis on an ABI 3130xl Genetic Analyzer. Fragments were scored using GeneMapper 4.0 (ABI). GDA (Lewis and Zaykin 2001) was used to calculate observed and expected heterozygosities, allelic richness, and to check for deviations from Hardy–Weinberg Equilibrium (HWE) using Fisher's exact test with 10,000 permutations.

The 37 loci showed high genetic diversity in *N. subnodosus* with 5–27 alleles per locus (avg = 15.3) and average expected heterozygosity of 0.76 (Table 1). Ten loci deviated significantly from HWE, all in the direction of heterozygote excess. Though other explanations are possible this is likely due to null alleles, prevalent in marine mollusks (Hedgecock et al. 2004; McGoldrick et al. 2000; Reece et al. 2004), and observed in prior parentage analysis of this species (Petersen et al. 2008). Cross amplification was not observed in *A. ventricosus* but was successful at 16 loci in *N. nodosus* and five and two loci, respectively, in *A. irradians* and *A. purpuratus* (Table 2).

Table 2 Results of cross-amplification (N = 3 individuals) showing only the loci where amplification was observed

Locus	*A. irradians*	*A. purpuratus*	*N. nodosus*
Nsub2A1B12	–	–	2 (166, 174)
Nsub2A1D12		1 (255)	6 (540–595)
Nsub2A1F01	U	–	5 (202, 294)
NsubA1A01	–	–	U
NsubA1B04	2 (515, 517)	–	6 (321–381)
NsubA1B09	–	–	2 (211, 234)
NsubA1B12	–	–	4 (202–294)
NsubA1C07	–	–	2 (136, 145)
NsubA1G09	2 (460, 479)	–	–
NsubA1H09	–	–	1 (300)
NsubA2C01	U	–	U
NsubA2D10	–	–	1 (271)
NsubA2F04	U	–	–
NsubA2H05	–	–	4 (415–426)
NsubB1F02	–	–	U
NsubB1G03	–	–	2 (323, 335)
NsubB207	–	3 (361–396)	4 (361–399)
NsubB2C05	–	–	4 (334–368)

Given are the number of alleles observed and their size range. No amplification was present in *A. ventricosus* (data not shown)

– indicates no amplification

U indicates unspecific amplification

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References

Hedgecock D, Li G, Hubert S, Bucklin K, Ribes V (2004) Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. J Shellfish Res 23:379–385

Ibarra AM, Petersen JL, Famula TR, May B (2006) Characterization of 35 microsatellite loci in the Pacific lion-paw scallop (*Nodipecten subnodosus*) and their cross-species amplification in four other scallops of the pectinidae family. Mol Ecol Notes 6:153–156

Kolpakov R, Bana G, Kucherov G (2003) mreps: efficient and flexible detection of tandem repeats in DNA. Nucleic Acid Res 31:3672–3678

Lewis PO, Zaykin D (2001) Genetic data analysis: computer program for the analysis of allelic data. http://lewis.eeb.uconn.edu/lewishome/software.html

McGoldrick DJ, Hedgecock D, English LJ, Baoprasertkul P, Ward RD (2000) The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster *(Crassostrea gigas)*: selection and null alleles. J Shellfish Res 19:779–788

Petersen JL, Ibarra AM, Ramirez JL, May B (2008) An induced mass spawn of the hermaphroditic lion-paw scallop, *Nodipecten subnodosus:* genetic assignment of maternal and paternal parentage. J Hered 99:337–348

Reece KS, Ribeiro WL, Gaffney PM, Carnegie RB, Allen SK Jr (2004) Microsatellite marker development and analysis in the Eastern oyster *(Crassostrea virginica)*: confirmation of null alleles and non-Mendelian segregation ratios. J Hered 95:346–352

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Methods in Molecular Biology. Humana Press, Totowa, p 365

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234