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PRIMER NOTE

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

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Abstract

Four microsatellite-enriched DNA libraries yielded 35 microsatellite loci from 100 primer pairs designed for Pacific lion-paw scallop, *Nodipecten subnodosus*. The number of alleles ranged from four to 28. Three of the 35 loci were not in Hardy–Weinberg equilibrium and linkage disequilibrium was found for one pair of loci. These microsatellites will be used to analyze the population structure of the species in Mexico's Baja Peninsula to propose management strategies for scallop aquaculture development. Twenty-six primer pairs cross-amplified in *Nodipecten nodosus*, whereas none (*Argopecten ventricosus*) or few cross-amplified in the *Argopecten* species.

Keywords: *Argopecten*, Guerrero Negro — Ojo de Liebre lagoon, Microsatellite, Primers

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The lion-paw scallop, *Nodipecten* (*Lyropecten*) *subnodosus*, is the largest scallop species found in the tropical waters of the East Pacific (Peña 2001). In Mexico, it is harvested on the Pacific and Gulf of California sides of the Baja California Peninsula and on the coast of Sonora state. The largest population is found in the lagoon of Ojo de Liebre, which is part of the Guerrero Negro lagoon system on the Pacific side of the Baja Peninsula. A fundamental question for the optimal development of lion-paw scallop aquaculture is whether populations native to different lagoons are genetically distinct. We isolated and characterized microsatellite loci that will allow for the analysis of population structure, from which optimum management strategies for aquaculture ventures can be proposed. These markers will also allow for evaluation of optimum spawning strategies for hatcheries that minimize genetic drift and inbreeding in this functional hermaphrodite species.

Whole genomic DNA was extracted using QIAGEN's DNeasy Tissue Kit from adductor muscle of wild lion-paw scallops naturally settled at Bahia de La Paz. DNA obtained from four scallops and 18 crayfish, the last also being studied in the Genomic Variation Laboratory (GVL), were mixed in equal concentrations prior to library construction. Four libraries enriched for tetra- and trinucleotide repeat motifs $(TAGA)_n$, $(TGAC)_n$, $(TACA)_n$ and $(ATC)_n$ were constructed, screened and sequenced (n = 170) from this mixed DNA by Genetic Identification Services (Chatsworth) according to Meredith & May (2002) and Schwartz & May (2004). One hundred and four primers were designed using PRIMERSELECT 4.0 (DNA Star Inc.) at the GVL of University of California-Davis.

To determine if loci amplified in scallop or crayfish, primers were first tested on four scallops from the same wild population. All 35 'positive' primers for the scallop were screened on 30 individuals, including 25 from Ojo de Liebre lagoon on the Pacific side of the Baja California Peninsula, and five from Bahia de La Paz, on the Gulf of California side of the peninsula. We further tested these primers for cross-amplification of the microsatellite loci in five to six individuals of each of the following scallop species: *Argopecten irradians* (USA, west Atlantic), *Argopecten purpuratus* (Chile, east Pacific), *Argopecten ventricosus* (Mexico, east Pacific) and *Nodipecten nodosus* (Venezuela, west Atlantic). DNA was extracted from muscle tissue preserved in 70% alcohol (*A. irradians* and *A. ventricosus*), or from dried adductor muscle tissue (*A. purpuratus* and *N. nodosus*).

Polymerase chain reaction (PCR) was performed using 5 ng of genomic DNA, 1 × *Taq* DNA polymerase buffer B, 2.0 mm $MgCl_2$, 0.2 mm of each dNTP, 1 μm of each primer and 0.38 U *Taq* DNA polymerase (all reagents from Promega), with a total PCR volume

of 10 μL. PCR was carried out using an MJ Research PTC-100 under the following conditions: 94 °C for 2.30 min, 30 cycles at 94 °C for 30 s, 56 °C for 30 s (53 °C for cross-amplifications), 72 °C for 30 s, followed by 72 °C for 5 min, and held at 4 °C. For those primers producing multiple-bands in the primary species (*N. subnodosus*), we performed touchdown PCR: 95 °C for 1 min followed by 30 cycles at 95 °C for 1 min, 67 °C for 45 s with a 0.5 °C decrease each cycle, and 72 °C for 2 min, using the same reagents and equipment. The amplified products were diluted 1:1 with 98% formamide loading buffer, denatured at 95 °C for 2 min and chilled on ice for 2–3 min. Samples were separated on a 5% denaturing polyacrylamide gel at 50 W for 70 min. Amplified products were visualized using the Sybr-Green™-agarose overlay protocol (Rodzen et al. 1998) and scanned with a Molecular Dynamics FluorImager 595. Product sizes were estimated by comparison with a standard 400 bp ladder (The Gel Company).

The primer sequences amplifying in *N. subnodosus*, their polymorphism data and analyses for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD), are presented in Table 1. The repeat motifs are specific to the one sequenced clone for each locus. Observed and expected heterozygosities were calculated from the 25 individuals from Ojo de Liebre lagoon. For the five individuals from Bahía La Paz, we present number of alleles and their size ranges. Deviations from HWE and LD were calculated using gda program (Lewis & Zaykin 2001), and significance was evaluated with the Fisher's exact test. Three loci were not in HWE after applying a Bonferroni correction, *NsubA007*, *NsubA010* and *NsubB215*, although it is not known if these deviations are due to null alleles or nonrandom mating because of the self-fertilizing capability for this functional hermaphrodite. Significant pairwise LD was found only between loci *NsubA223* and *NsubC262* following a test using genotypes to prevent within locus disequilibrium from affecting the significance and a Bonferroni correction for multiple comparisons ($P = 0.0014$).

Results of cross-amplification of primers on the other four species are in Table 2. Of the 35 primer pairs developed, nine (25%) resulted in no amplification for all the cross-amplified species. None of the primers amplified in *A. ventricosus*. Twenty-six (74%) of the 35 primer pairs that successfully amplified polymorphic loci in *N. subnodosus* amplified in *N. nodosus*, the Atlantic species from the same genus. The next largest cross-amplification was seen for *A. irradians* (11 primer pairs) and for *A. purpuratus* (seven primer pairs).

Table 1 Characterization of 35 microsatellite loci in lion-paw scallop (*Nodipecten subnodosus*) from Lagoon Ojo de Liebre (OL) within the Guerrero Negro lagoons system and Bahia de La Paz, Mexico. GenBank Accession nos, primer sequences, repeat motif, loci amplified using touchdown PCR, number of individuals genotyped, number of alleles, gel estimated allele size range (bp), observed and expected heterozygosities, and test for conformance to Hardy-Weinberg equilibrium for the Ojo de Liebre lagoon. Allele number and size ranges for Bahía de La Paz

Locus	GenBank Accession no.	Primer sequence (5'-3')	TD PCR	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	H_O	H_E	P(HWE) & significance[(1)]	Bahía de La Paz ($n = 5$) no. of alleles (size range)
NsubA001	DQ108619	**F:** GAGGATGACAGTGTGAAGATG **R:** CACAAAACAGAATGTGTGAAGA	+	23	$(TGA)_3$ $TTA(TGA)_9$	4	285–300	0.65	0.54	0.6166	4 (285–300)
NsubA004	DQ108620	**F:** TTTGACAGACATCCCTTCTTAC **R:** GAATGCGACATAGACATCG	+	21	$(TCA)_{10}$	9	230–267	0.86	0.86	0.7844	4 (237–247)
NsubA005	DQ108621	**F:** CGACAAACATCCCCTCTT **R:** ACAGCGACCAGTGTAACG	+	25	$(TCA)_{12}$ $AAATCA$	4	204–218	0.80	0.66	0.2937	5 (204–218)
NsubA007	DQ108622	**F:** AACGCTGTCACTGAAACAAAAG **R:** TCGGTGATACAAGTTGAAGAGG	+	21	$(CAT)_{18}(GTC)_5$ $CAT(CGT)_4(C)_{11}$	13	159–285	0.48	0.91	0.0000*	5 (200–242)
NsubA010	DQ108623	**F:** TTTGACTTGAAAGACCATCTCT **R:** CGGCACATCATTGTTACTAAA	+	22	$(TCA)_2N_{10}$ $TCAATCATACA(TCA)_3$	6	126–144	0.95	0.74	0.0013*	4 (128–138)
NsubA208	DQ108624	**F:** GTTACGTGCGTGGACTGAA **R:** CGGCATCTGTACCTGACAC	+	24	$(ATG)_9$	4	283–298	0.58	0.54	0.8284	4 (287–298)
NsubA214	DQ108625	**F:** CCTCCTCTCCACTTTCTTTCAC **R:** CACCCGGAGCTTAAATATAAGG		22	$(TCA)_{10}$ $TA(TCA)_{11}$	5	235–290	0.50	0.67	0.0069	3 (235–278)
NsubA222	DQ108626	**F:** TTTTGTGACGCACCAACTA **R:** GACAGGGGACCAGTCTACC		22	$(TCA)_{21}(TCG)_5(TCA)_2$ $TCG(TCA)_3(TCG)_9$	9	220–320	0.91	0.80	0.6237	5 (220–300)
NsubA223	DQ108627	**F:** CCTGCTCTTTGAACTGCTAG **R:** CTGTTGCTGTTCTCAGACAAC		24	$(CAT)_5TAT(CAT)_6$ $CAA(TCA)_{15}$	14	120–240	0.79	0.90	0.1053	8 (120–200)
NsubA227	DQ108628	**F:** ACCTCATCTGGTGTTCAATTC **R:** CAATTTCCAGTGTGTTGTCAT		21	$(ATC)_{21}(TCG)_{12}$	13	180–300	0.95	0.92	0.6134	5 (180–300)

Table 1 Continued

Locus	GenBank Accession no.	Primer sequence (5′-3′)	TD PCR	*n*	Repeat motif	No. of alleles	Gel estimated allele size range (bp)	H_O	H_E	P(HWE) & significance[1]	Bahía de La Paz (*n* = 5) no. of alleles (size range)
NsubA229	DQ108629	**F**: TCTCCCTGGAATGATAAAGG **R**: GGCTGTAAATCTCACCAAATG		19	$(ACG)_5N_9(ATG)_4A(CGA)_8(TGA)_6$	7	242–284	1.00	0.83	0.3769	4 (242–280)
NsubA231	DQ108630	**F**: CTGGGAATTTGTAGCTTTACC **R**: GAGGAAGATGATGATGATGATT	+	25	$(TCA)_5TCTCTTAAA(TCA)_4$	4	138–150	0.48	0.57	0.1647	3 (138–147)
NsubA235	DQ108631	**F**: GTGGCAAGTCTTATTTGGTAGA **R**: CTGTTTACTTTCGATGCAAGTT		24	$(TGA)_{11}$	7	200–230	0.71	0.78	0.4853	5 (200–225)
NsubA238	DQ108632	**F**: TTTTATCGAAATGTCGGATGTC **R**: TGCTGGTCGAGTTCTTAAAGAG		24	$(TCA)_4N_7(TCA)_8N_6(TCC)_8$	13	98–155	1.00	0.92	0.5319	6 (110–155)
NsubA243	DQ108633	**F**: CGCAGGAAACGATCATTTAGTA **R**: CACACGGAACATCAACTTAACC	+	25	$(GAT)_7AT(GAT)_{11}$	7	142–165	0.88	0.78	0.0078	5 (142–158)
NsubA245	DQ108634	**F**: TGCCTATTTCGATAACTGATTG **R**: TAATGCTAATACGGCACCAGT		25	$(TCA)_8$	6	293–320	0.88	0.81	0.2313	4 (305–320)
NsubA249	DQ108635	**F**: CCGCTGAAAATCTCTCCT **R**: GCCCCATCGTAACAATCT	+	21	$(TCA)_{13}$	8	240–295	1.00	0.85	0.1025	6 (240–278)
NsubA261	DQ108636	**F**: ACGAAAAGTTGAAATCGTGATC **R**: CGTTTATACAGGCATGTTTCTG	+	22	$(CAT)_6T(ATC)_6(GTC)_{11}$	9	240–320	0.95	0.87	0.6572	4 (240–280)
NsubA262	DQ108637	**F**: ATCACGAGTCAAAACAGTATCG **R**: CCTGGTAGGTGAATCACACTAA		20	$(ATC)_4N_{18}(ATC)_8$	12	285–430	0.70	0.89	0.0084	6 (290–405)
NsubA266	DQ108638	**F**: GCCAAATTATGAGACATCCAC **R**: AATGGGTTGATGATGATTGTC	+	21	$(TCA)_9G(CAT)_3$	14	132–225	0.90	0.89	0.5334	6 (147–210)
NsubA274	DQ108639	**F**: GAACTTGGTGCAGTTATCTTCA **R**: ATAGCGTAAAGTGTGCAACGT	+	22	$(TCA)_9$	12	260–325	0.95	0.87	0.5650	6 (262–290)
NsubB007	DQ108640	**F**: GGCATGTGAACTACATTTCAAT **R**: TTCCATTCCGTTTACACAGATA		22	$(ATGT)_{12}$	8	260–320	0.77	0.83	0.5241	5 (270–295)
NsubB210	DQ108641	**F**: GAGAGGTGAAGTGAAAGTGAAG **R**: TGGAAGTACAGAAAGGAATGTC		20	$(CATA)_{12}$	5	240–290	0.60	0.76	0.0016	5 (240–290)
NsubB215	DQ108642	**F**: CCTGACTTATCCTGTCATTTCA **R**: TATTCCTTTCTCGCCCATAT		25	$(ATAC)_{13}N_6(ATAC)_4N_6(ATAC)_{15}$	28	95–420	0.80	0.97	0.0000*	9 (95–400)
NsubB235	DQ108643	**F**: GTCCTTTGGTCTTGGTTACTG **R**: ATGGATTTGACATCATCACAAC		22	$(TACA)4CA(TACA)_5TACG(TACA)_3$	5	145–180	0.86	0.75	0.3788	5 (145–180)
NsubB252	DQ108644	**F**: ACAAGCGAATACAACGACA **R**: ACCATTGACCAGGCTACAT	+	22	$(TGTA)_4TGGTA(TGTA)_9$	4	150–178	0.73	0.72	0.5375	4 (150–178)
NsubB278	DQ108645	**F**: CACACGATTCACTTTCCTATTG **R**: GCTTCAGCTTAACGATTGAAAC	+	19	$(TACA)_{12}CA(TACA)_{13}CA(TACA)_{11}$	17	138–440	1.00	0.93	0.9794	6 (142–358)
NsubC020	DQ108646	**F**: CACCTACCTCTAGGTGTGGAAG **R**: TGTGAAAGACAGAGTTATGATCGAG		21	$(TGTC)_4ATGTCTGACC(TATC)_7$	10	195–280	0.81	0.88	0.2491	8 (195–270)
NsubC023	DQ108647	**F**: CCCCAAAACACAATGCAGAACAAA **R**: TTATGGCCGGAAAGTATCAATCAG		23	$(TAGA)_{16}(TGGA)_2TGA(TGGA)_8(TAGA)_2(AGA)_{16}$	20	250–520	0.96	0.96	0.5822	4 (360–500)
NsubC205	DQ108648	**F**: GTCAAAAGGTTGGCAGTCTAAG **R**: CAGCATAAACCCTACTCATGGT	+	21	$(GATA)_{10}$	9	195–240	0.95	0.84	0.6253	2 (195–202)
NsubC218	DQ108649	**F**: CCGCAAATATCATTTATCTCG **R**: GTCGTTAAAGGTTGGTGTTG	+	18	$(GTCT)_4(ATCT)_{14}$	9	170–320	0.94	0.89	0.4478	7 (170–290)
NsubC250	DQ108650	**F**: ACCTGAAGCAAAGAAAGAAAGA **R**: TTACCTGTAGGTGTGGAGACAC		25	$(GACA)_4(GATA)_4CATA(GATA)_4$	11	250–320	0.84	0.89	0.8634	8 (255–300)
NsubC261	DQ108651	**F**: TTCAAACGCACGTCATTTG **R**: CTTGGCACCATATTCGGAC		24	$(TATC)_8$	9	125–200	0.71	0.85	0.0031	4 (145–160)
NsubC262	DQ108652	**F**: CCGCAAATATCATTTATCTCG **R**: GCATTGTCAACTGAATTAGCA		23	$(TGTC)_4(TATC)_{10}$	15	155–250	0.74	0.93	0.1219	4 (168–217)
NsubC275[2]	DQ108653	**F**: AGGCTGGGGTCACTATCA **R**: CGGTGATTACACAAATACACC	+	22	$(TATC)_7$	5	78–114	0.86	0.72	0.5216	3 (78–90)

+ Best amplification — resolution by using TD PCR.
*Statistically significant ($P < 0.05$).
(1) After standard Bonferroni correction.
(2) Forward primer within minisatellite; amplification at 3 other size ranges (c. 130–150, 200–220 & 250–270).

Table 2 Cross-species amplification results (number of alleles per locus with size range (bp)) in parentheses of 35 microsatellite loci for the scallop family Pectinidae, genus *Argopecten* (*A. irradians*, *A. purpuratus*, *A. ventricosus*) and *Nodipecten* (*N. nodosus*)

Locus ID	*A. irradians* (*n* = 6) USA — Atlantic	*A. purpuratus* (*n* = 5) Chile — Pacific	*A. ventricosus* (*n* = 6) Mexico — Pacific	*N. nodosus* (*n* = 6) Venezuela —Atlantic
NsubA001	U	—	—	U (140, 215)
NsubA004	—	—	—	2 (215–235)
NsubA005	—	—	—	3 (180–200)
NsubA007	U	U	—	6 (160–220)
NsubA010	U (115, 300)	—	—	4 (120–135)
NsubA208			—	3 (280–300)
NsubA214	—	—	—	—
NsubA222	—	—	—	—
NsubA223	U	—	—	7 (90–120)
NsubA227	—	—	—	U (200–240)
NsubA229	—	—	—	—
NsubA231	4 (300–400)	U (340, 460)	—	5 (140–150)
NsubA235	—	6 (200–300)	—	7 (200–235)
NsubA238	—	—	—	U (120–140)
NsubA243	—	—	—	U (120–160)
NsubA245	—	—	—	—
NsubA249	—	—	—	2 (250–270)
NsubA261	—	—	—	7 (220–300)
NsubA262	—	U	—	5 (280–340)
NsubA266	—	—	—	—
NsubA274	U	—	—	3 (260–280)
NsubB007	—	—	—	—
NsubB210	—	—	—	—
NsubB215	—	—	—	5 (100–300)
NsubB235	—	—	—	U
NsubB252	U	—	—	2 (140–160)
NsubB278	—	—	—	—
NsubC020	—	—	—	2 (160–200)
NsubC023	—	—	—	—
NsubC205	2 (> 400)	U	—	3 (170–210)
NsubC218	U	U	—	4 (290–320)
NsubC250	2 (300–340)	—	—	4 (210–240)
NsubC261	—	—	—	3 (140–240)
NsubC262	—	—	—	6 (150–250)
NsubC275	U	U	—	U
Total no. of amplified loci	11	7	0	26

U, indicates amplification but unclear; '—' indicates no amplification; (num-num) indicates range; (num, num) indicates when two bp ranges amplified.
Species, sample size (*n*), and origin — distribution.

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