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)\textsubscript{n}, (TGAC)\textsubscript{n}, (TACA)\textsubscript{n}, and (ATC)\textsubscript{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...
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 (HWEpv).

| Locus & GenBank Accession | Repeat Motif | Forward Primer (5′-3′) | Reverse Primer (5′-3′) | Primer Label, PCR Profile | N | A | (Range) | H_e | H_o | HWEpv |
|--------------------------|--------------|------------------------|------------------------|---------------------------|---|---|--------|-----|-----|--------|
| *Nsub2A1A06* | (ATAG)_5AGG(TAGA)_7TA | TTTCCCTTTTGCAAAAACATC | | IV | 24 | 10 | | 0.87 | 0.96 | 0.753 |
| FIJ986332 | | | | | | | | | | |
| *Nsub2A1B12* | (ATCT)_3ATC | GCCCTCTGAACCAAAACACAT | | IV | 19 | 12 | | 0.82 | 0.47 | 0.008 |
| FIJ986334 | | | | | | | | | | |
| *Nsub2A1C06* | (TCTA)_1TCT...C_18...(TC)_7...(CA)_9 | CTATTTGTTGGTGGGGGTGT | | I | 6 | 5 | | 0.79 | 0.67 | 0.018 |
| FIJ986333 | | | | | | | | | | |
| *Nsub2A1D12* | (TACTC)_1(CA)_13(TACA)_8T | GACAAACAAAAAGGCTGAT | | III | 21 | 25 | | 0.97 | 0.67 | 0.000* |
| FIJ986335 | | | | | | | | | | |
| *NsubA1A01* | (GAT)_9 | CGGCCAGTGAACGGTATAG | | II | 24 | 13 | | 0.88 | 0.58 | 0.002* |
| FIJ986338 | | | | | | | | | | |
| *NsubA1B04* | (ATGG)_3ATG... (GAT)_3(TGA)_7T | AGCAGAGGAAGCGATGCCTT | | III | 22 | 20 | | 0.92 | 1.00 | 0.707 |
| FIJ986339 | | | | | | | | | | |
| *NsubA1B09* | (CAT)_14 | TTGCATACGAACTGCTACT | | II | 24 | 15 | | 0.93 | 0.96 | 0.947 |
| FIJ986340 | | | | | | | | | | |
| *NsubA1B12* | (TCC)_2T(N)_6(CAT)_17(TCA)_15GT | GCTGGGCATTCCGTTTTCAGA | | III | 24 | 24 | | 0.96 | 1.00 | 0.313 |
| FIJ986341 | | | | | | | | | | |
| *NsubA1C02* | (TCA)_8TC | TTGCATAAGGTTCTGTTTTCTG | | III | 22 | 18 | | 0.94 | 0.68 | 0.001* |
| FIJ986342 | | | | | | | | | | |
| *NsubA1C07* | (ATC)_13A(ACA)_3AC | CTATAACCGCCCTCAGCATCTC | | I | 22 | 21 | | 0.95 | 0.68 | 0.000* |
| FIJ986343 | | | | | | | | | | |
| *NsubA1C08* | (TGA)_5T | AGGCGAATAATCGAGTCTG | | II | 24 | 11 | | 0.86 | 0.67 | 0.076 |
| FIJ986344 | | | | | | | | | | |
| *NsubA1C12* | (TGA)_5TG | ACTGCAACACAAACATGGA | | I | 20 | 6 | | 0.76 | 0.80 | 0.667 |
| FIJ986345 | | | | | | | | | | |
| *NsubA1D10* | (TGA)_3T | CCTCCAGGCTCATGTTTCTTC | | III | 21 | 8 | | 0.85 | 0.48 | 0.000* |
| FIJ986346 | | | | | | | | | | |
| *NsubA1F03* | (ATG)_3AT | ACAATGGCGGATATGACGG | | IV | 23 | 11 | | 0.69 | 0.57 | 0.083 |
| FIJ986347 | | | | | | | | | | |
| Locus & GenBank Accession | Repeat Motif<sup>a</sup> | Forward Primer (5'-3') | Reverse Primer (5'-3') | Primer Label<sup>b</sup>, PCR Profile<sup>c</sup> | N | A (Range) | H<sub>e</sub> | H<sub>o</sub> | HWE<sub>pv</sub> |
|---------------------------|------------------------|------------------------|------------------------|-----------------------------------------------|---|----------|-------|-------|-----------|
| *NsubA1F04* FJ986348     | (TGA)<sub>10</sub>(N)₆(TGA)<sub>4</sub> | AAGACCGCAGCTTATCAT     | AAATCAGCTCGGCTTCTCT     | I                                            | 24 | 18       | 0.92  | 0.79  | 0.036     |
| *NsubA1F07* FJ986349     | (GAT)<sub>12</sub>(GAT)<sub>9</sub>G | TGTTGATAAGAATACCTTGAGAT | CACCCTCAACCACAAATCTA    | II                                           | 24 | 18       | 0.93  | 0.83  | 0.116     |
| *NsubA1F12* FJ986350     | (GAT)<sub>12</sub>(GAT)<sub>9</sub>G | GATGCAAAAACATCGCAAG     | GCTTCTTATATTCATATTGGA   | II                                           | 24 | 17       | 0.91  | 0.92  | 0.858     |
| *NsubA1G09* FJ986351     | (CAT)<sub>9</sub>C     | GCTATCGCTGGTCGGTGCGAACA| TGGGGAATCTCCTGATGT      | IV                                           | 24 | 14       | 0.91  | 0.92  | 0.867     |
| *NsubA1H09* FJ986352     | (ATC)<sub>20</sub>A   | AAGGAAGCAATTTTATGAATCGTG | AATGTTGGCTAGGCGAGAT     | IV                                           | 24 | 13       | 0.87  | 0.92  | 0.186     |
| *NsubA1H12* FJ986353     | (ATG)<sub>10</sub>    | GGTATGGTGGTCGTTCTGTA   | CTGCCGTAGCGGAGAGAT      | IV                                           | 24 | 14       | 0.90  | 0.88  | 0.822     |
| *NsubA2C01* FJ986354     | (TGA)<sub>10</sub>(CGA)<sub>10</sub>C | AGAGTTTGCGTAAAGGCAGAT | GGAATGGTTGCTCAGCTCTCA  | IV                                           | 22 | 21       | 0.94  | 0.91  | 0.476     |
| *NsubA2D10* FJ986355     | (TCA)<sub>12</sub>T   | TTTCCATCAACACAGCAAGCA  | CCCATGTATGATGCTGCTG     | I                                            | 21 | 5        | 0.56  | 0.57  | 0.284     |
| *NsubA2F04* FJ986356     | (CGT)<sub>12</sub>(TCA)<sub>12</sub>T | ATTTGTTGGTGGTGTCAGTG  | GTGTTTAAATGCGGAGCTG    | I                                            | 24 | 17       | 0.91  | 0.92  | 0.561     |
| *NsubA2F08* FJ986357     | (TGA)<sub>7</sub>     | CGATGAGCGTCCACAGGAAGT  | GCAAGCTTCTTATAGGCTGAG  | IV                                           | 22 | 10       | 0.86  | 0.50  | 0.001*    |
| *NsubA2H05* FJ986358     | (ATG)<sub>10</sub>ATA(ATG)<sub>7</sub>A | GCTATGCGAGAAGCTAAGG     | GTGCCGTGCGTATGCTGCT    | III                                          | 24 | 9        | 0.83  | 0.88  | 0.925     |
| *NsubB1A02* FJ986359     | (CAT)<sub>7</sub>(ACAT)<sub>17</sub> | CATCCCTCCATCCAATCAGT   | GTGATCTCCACAGGGAAGG     | IV                                           | 19 | 14       | 0.88  | 0.79  | 0.031     |
| *NsubB1C05* FJ986360     | (GT)<sub>12</sub>GAGG(CTAT)<sub>10</sub>B | CCCTGATGCTAGCCTGCAAA   | GCACCGATGCTATGCTGTTCC  | I                                            | 20 | 19       | 0.95  | 0.95  | 0.576     |
| *NsubB1C07* FJ986361     | (TATG)<sub>12</sub>(ATG)<sub>15</sub>(ATG)<sub>13</sub>ATG | ACAGGTCTTCGAGTTTTCTGC  | CCTGGATTTGCGCAATTTTATG | II                                           | 24 | 27       | 0.96  | 0.50  | 0.000*    |
| *NsubB1E11* FJ986362     | (ATAC)<sub>13</sub>...(ATAC)<sub>14</sub>A(NG)<sub>7</sub>(CGTG)<sub>3</sub>C | TACGTTACGGCCACCTACAA  | GCCGCCATATGGAAGCAGACA  | II                                           | 21 | 22       | 0.95  | 1.00  | 0.547     |
| *NsubB1F02* FJ986363     | (ACCT)<sub>14</sub>AC(ATA)<sub>10</sub>A(CATA)<sub>15</sub>CA (ACAT)<sub>5</sub>ACA | GCTATTTTTTGCTGGTGGTG   | TGGCCACATGTAAGACGACA   | I                                            | 21 | 16       | 0.91  | 0.52  | 0.000*    |
| *NsubB1G03* FJ986364     | (GTAT)<sub>9</sub>A(TATG)<sub>13</sub>T | TTGCACTGACTATGTAATTTTG | AAGAGGAATGGTGGCTGCGTG  | IV                                           | 19 | 17       | 0.94  | 0.89  | 0.361     |
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 MgCl₂, 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, Tₐ for 90 s, and 72°C for 1 min, additional cycles of 94°C for 45 s, Tₐ for 90 s, and 72°C for 1 min, and a 10 min final extension at 72°C (see Table 1 for Tₐ 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 percentage 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).
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Table 2

| 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