Title	Genetic structure of the Peruvian scallop <i>Argopecten purpuratus</i> inferred from mitochondrial and nuclear DNA variation
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Citation	Marine Genomics, 9, 1-8 https://doi.org/10.1016/j.margen.2012.04.007
Issue Date	2013-03
Doc URL	http://hdl.handle.net/2115/52046
Type	article (author version)
File Information	Genetic structure of the Peruvian scallop <i>Argopecten purpuratus</i> inferred from nuclear and mitochondrial DNA variation.pdf

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1 **Genetic structure of the Peruvian scallop *Argopecten***
2 ***purpuratus* inferred from mitochondrial and nuclear DNA**
3 **variation**

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Abstract The population genetic structure of the Peruvian scallop *Argopecten purpuratus* from three different wild populations along the Peruvian coast was analyzed using nine microsatellite loci and a partial region (530 bp) of the mitochondrial 16S rRNA gene. A total of 19 polymorphic sites in the 16S rRNA gene defined 18 unique haplotypes. High genetic diversity was presented in all populations. Statistical analysis of mitochondrial DNA revealed no significant genetic structure ($\Phi_{ST} = 0.00511$, $P = 0.32149$) among the three localities. However, microsatellite analysis showed low (2.86%) but highly significant ($P=0.0001$) genetic differentiation among populations, most of the variation was found in Independencia Bay population, which is located in the Peruvian National Reserve of Paracas. Neutrality tests based on mitochondrial haplotypes were performed to assess signatures of recent historical demographic events. Overall results from Tajima's D and Fu's F_S tests indicated significant deviations from neutrality. To our knowledge, this study constitutes the first investigation based on mitochondrial and microsatellite markers on the genetic structure of *A. purpuratus*.

Keywords Pectinidae • Scallop • Peru • Microsatellites • Genetic diversity

1. Introduction

The Peruvian scallop, *Argopecten purpuratus* (Lamarck, 1819), which is a marine bivalve belonging to the family Pectinidae, is naturally distributed along the Pacific coast from Paita, Peru to Tongoy in Chile (Wolff and Mendo, 2000). It is the most economically important mollusk in Peru and during the year 2010 the total export of *A. purpuratus* from Peru reached US\$119 million (PROMPERU, 2011). Following El Niño events in 1982-1983, *A. purpuratus* populations in southern Peru proliferated notably due to several factors including increased growth rate and larval survival, improved recruitment and reduced mortality from predation (Arntz et al., 2006). Consequently, both fishery and mariculture activities increased remarkably which caused the beginning of exportation activities (Bandin and Mendo, 1999). *A. purpuratus* stocks were overexploited afterward, due mainly to a high fishing pressure and to environmental conditions normalization (Mendo et al., 1988). In Peru, there are only four scallop hatcheries (three private and one government-run), so the aquaculture industry acquires most of its seeds from the wild.

Seed translocation activities have been reported in this species, especially after El Niño events (Mendo et al., 2008). Translocation of individuals between different populations may result in outbreeding depression, loss of local adaptation, replacement of recipient genetic background and disease transmission (Weeks et al., 2011). Genetic effects due to artificial spat relocation have been studied in bivalves (Arnaud-Haond et al., 2003; Beaumont, 2000; Gaffney et al., 1996; Vilella et al., 1998). It is well known that most marine species have a planktonic larval stage, which may result in a high gene flow

81 between populations due to passive larval dispersal by marine currents (Cho et al., 2007;
82 Katsares et al., 2008). Most of such marine species are thought to have little genetic
83 structure (Yuan et al., 2009). However, extrinsic factors including marine currents and
84 gyres, hydrographical barriers to dispersal, bays and islands, and even anthropogenic
85 activities can affect the population structure of several marine bivalves (Ni et al., 2011;
86 Zhan et al., 2009a). Significant genetic differentiation over small geographic scales has
87 been revealed in clams, mussels, and scallops (Luttikhuizen et al., 2003; Ni et al., 2011;
88 Ridgway, 2001; Zhan et al., 2009a).

A. purpuratus is a
89 continuous spawner species with spawning peaks during late summer and autumn (Wolff,
90 1988) and its larval stage has been reported to last between 16 to 25 days under hatchery
91 conditions (von Brand et al., 2006). Thus, due to its high dispersal potential, this species
92 is expected to have little or no genetic structure. Nevertheless, in spite of its great
93 commercial and ecological values, so far little is known about the genetic variation and
94 population structure of *A. purpuratus* stocks.

To date, population genetic
95 studies have not been conducted in *A. purpuratus* except for classic approach using
96 allozymes as genetic markers. For example, Galleguillos and Troncoso (1991) found no
97 genetic differences among Chilean populations using allozyme electrophoresis. However,
98 Moragat et al. (2001), using allozymes and morphological characters detected statistically
99 significant differences between two *A. purpuratus* populations from northern Chile.
100 These contrasting results based both on allozyme analyses could be attributed to the
101 different number and polymorphic loci found and analyzed in each study and also to the
102 oceanographic geographical features of the surveyed bays.

103 Microsatellite and mitochondrial markers have been used to determine the

population structure in Pectinidae species including *Patinopecten caurinus* (Gaffney et al., 2010), *A. irradians* (Hemond and Wilbur, 2011), *Amusium pleuronectes* (Mahidol et al., 2007), *Mizuhopecten yessoensis* (Nagashima et al., 2005), *Nodipecten subnodosus* (Petersen et al., 2010), and *Chlamys farreri* (Zhan et al., 2009a). This is the first study based on mitochondrial and microsatellite markers on the population genetic structure of *A. purpuratus* from Peruvian localities.

2. Materials and methods

2.1 Sampling and DNA isolation

A total of 69 individuals of *A. purpuratus* were sampled from three of the main natural scallop locations along the Peruvian coastline: Sechura Bay (5° 40' S, 80° 53' W; population code: "SEB"), Samanco Bay (9° 14' S, 78° 30' W; population code: "SAB"), and Independencia Bay (14° 15' S, 76° 11' W; population code: "INB"). Approximately 200 mg of adductor muscle was dissected from each individual, preserved in 95% ethanol and stored at -20 °C. Genomic DNA was isolated from the adductor muscle following the standard phenol-chloroform protocol and adjusted to a concentration of 100 ng/μl and used for PCR. Figure 1 provides information about the sampling locations and sample size.

2.2 Complete mitochondrial 16S rRNA gene determination

In order to determine the most polymorphic region of the mitochondrial 16S rRNA gene in *A. purpuratus*, the full-length 16S rRNA gene sequence was determined in 15 individuals of *A. purpuratus* from three different localities by “primer walking” strategy. For this purpose, the 16S rRNA adjacent gene pairs (ND1 and COI) sequences from already developed scallop mitochondrial genomes (*A. irradians*, GenBank accession: DQ665851; *M. yessoensis*, GenBank accession: FJ595959; *P. magellanicus*, GenBank accession: DQ088274; *M. nobilis*, GenBank accession: FJ595958; and *C. farreri*, GenBank accession: EF473269) were multi-aligned using CLUSTAL W (Thompson et al., 1994), and two degenerated primers (ND1E-forward 5'-CGGCTTCGCCATGATCtTyatygnga-3' and CO1AB-reverse 5'-GGTGCTGGGCAGC-cayatnccngg-3') were designed using the CODEHOP strategy (Rose et al., 2003). Degenerated oligos in combination with the universal reverse 16S R (Puslednik and Serb, 2008) and forward primer 16Sar1 (Palumbi et al., 1991) produced a 1600 and 800 bp size product (covering the full 16S rRNA gene) respectively, with an overlapped sequence fragment. PCR reactions consisted of 100 ng of template DNA, 40 µM dNTPs, 1X Ex *Taq* buffer (TaKaRa), 0.5 µM each primer, 0.025 U Ex *Taq* polymerase (TaKaRa) in a total volume of 20 µl. Thermocycling conditions for primers ND1E-forward and 16S-R reverse were as follows: initial denaturation for 1 min at 94 °C, followed by 30 cycles of denaturation for 15 s at 94 °C, annealing for 30 s at 50 °C, and extension for 90 s at 72 °C, followed by a final extension for 10 min at 72 °C; and conditions for primers 16Sar1-forward and CO1AB-reverse: initial denaturation for 1 min at 94 °C, followed by 30 cycles of denaturation for 15 s at 94 °C, annealing for 30 s at 55 °C, and extension for 3

min at 72 °C, followed by a final extension for 10 min at 72 °C. The start and end of the 16S rRNA gene were determined with multiple sequence alignments of the same gene from other published bivalve mitochondrial genomes. The 16S rRNA gene full sequences obtained in the 15 individuals of *A. purpuratus* were multi-aligned using BioEdit (Hall, 1999) and the most polymorphic region was determined by calculating the entropy (Hx, as implemented in BioEdit, data not shown) which is low in conserved sites and high in variable sites (supplementary data Table 1 shows all primer sets used to develop the full 16S rRNA gene in *A. purpuratus*).

2.3 Mitochondrial 16S rRNA gene partial sequence amplification

The 16S rRNA gene polymorphic region was amplified in 68 scallop samples using the primers: 16SAA-forward 5'-GGTCCCACCTAGAAGCTAATG-3' and 16SAA-reverse 5'-CCCGGAGTAACTTCTTCTACTA-3'. PCR conditions were as follows: 100 ng of template DNA, 40 µM dNTPs, 1X Ex *Taq* buffer (TaKaRa), 0.5 µM each primer, 0.025 U Ex *Taq* polymerase (TaKaRa) in a total volume of 20 µl and thermocycling condition: initial denaturation for 1 min at 94 °C, followed by 30 cycles of denaturation for 15 s at 94 °C, annealing for 30 s at 60 °C, and extension for 60 s at 72 °C, followed by a final extension for 10 min at 72 °C. All PCR products were sequenced in both directions using the same primers on an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystem). All haplotype-sequences have been deposited in

GenBank/DDBJ/EMBL DNA databases with accession numbers from JN848501 to JN848518.

2.4 Microsatellite genotyping

The genotypes of nine *A. purpuratus* trinucleotide microsatellite loci (see supplementary data Table 2) were determined as described in Marín et al. (2012). Amplified PCR products were sequenced using formamide and GeneScan LIZ-500 size standard (Applied Biosystems) in the ABI PRISM 3130XL Genetic Analyzer (Applied Biosystem) and GeneMapper 3.7 software (Applied Biosystems) was used for allele scoring.

2.5 Statistical analysis

2.5.1 Mitochondrial data

Mitochondrial 16S rRNA gene sequences were aligned using CLUSTAL W (Thompson et al., 1994). Levels of mtDNA diversity were assessed by calculating haplotype (h) and nucleotide diversity (π) with ARLEQUIN v. 3.5 (Excoffier and Lischer 2010). The same software was used to calculate pairwise haplotype divergences using the fixation index Φ_{ST} (Excoffier et al., 1992), which consider information on haplotype frequency and genetic distances. Using ARLEQUIN, we also perform an analysis of molecular variance (AMOVA) to estimate the amount of genetic variability within and among populations. AMOVA calculates the proportion of variation among groups (Φ_{CT}), among populations within groups (Φ_{SC}), and within populations (Φ_{ST}). For this analysis,

populations were grouped as a single gene pool, and also according to geographic regions considering the distance between populations (SEB+SAB, INB). The significance of both pairwise population comparisons and AMOVA Φ_{ST} were tested using 10000 permutations. In addition, a minimum spanning network showing the phylogenetic relationships among the different 16S rRNA gene haplotypes was constructed using ARLEQUIN software.

2.5.2 Microsatellite data

Microsatellite data were tested for departures of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium with GENEPOP software version 4.0.10 (Rousset, 2008). Significance of deviations from HWE expectations was determined using the Markov chain exact probability test (Guo and Thompson, 1992). Linkage disequilibrium among loci was assessed using Fisher exact test as implemented in GENEPOP. ARLEQUIN v. 3.5 (Excoffier and Lischer, 2010) was used to calculate the observed (H_O) and expected heterozygosity (H_E). Further, the software FSTAT 2.9.3.2 (Goudet, 1995) was used to estimate allelic richness per locus and sample (R_s). In all cases where multiple tests were performed, significance levels were adjusted using Bonferroni correction method (Rice, 1989). The MICRO-CHECKER 2.2.1 software (van Oosterhout et al., 2004) was used to check microsatellites for null alleles and scoring errors. Scallops with missing data at more than 2 loci were omitted for further analyses. ARLEQUIN 3.5 (Excoffier and Lischer, 2010) was also used to assess the level of genetic differentiation among localities by calculating pairwise

F_{ST} -values and an AMOVA was carried out to check for geographical genetic structure. Performance of AMOVA was assessed with exact test based on 10,000 permutations. To further assess genetic population structure, a Bayesian clustering analysis was performed using STRUCTURE v. 2.3.2 (Pritchard et al., 2000). The Bayesian clustering method assigns individuals into genetic clusters and can reveal cryptic population structure. To infer the number of genetic clusters, 10 independent runs of $K=4$ were performed at 1000000 Markov Chain Monte Carlo (MCMC) repetitions with a 100000 burn-in period using no prior information.

2.5.3 Demographic analyses

We used 16S rRNA mitochondrial gene data to infer patterns of demographic history in *A. purpuratus*. Neutrality tests based on the distribution of segregating sites (Tajima's D ; Tajima, 1989) and on the haplotype distribution (Fu's F_S ; Fu, 1997) were calculating using ARLEQUIN (using 10000 coalescent simulations) to assess signatures of past population expansion. Significant negative values of these parameters are indicative of an excess of low-frequency variants, which can result from populations that have undergone a recent expansion; in contrast, positive values reflect an excess of high-frequency derived mutations, which is a hallmark of positive selection (Barreiro and Quintana-Murci, 2010). To further investigate the possibility of population growth, pairwise mismatch distributions were calculating using DnaSP v. 5.10.01 (Librado and Rozas, 2009). The pattern of pairwise differences between haplotypes results in a unimodal mismatch distribution for expanding populations, whereas populations at

demographic equilibrium yield a multimodal pattern (Rogers and Harpending, 1992). Lastly, Harpending's raggedness index (Hri; Harpending, 1994), based on mismatch distributions, was calculated to test for deviations from the sudden expansion model. A combination of negative values of Tajima's D and Fu's F_S , and unimodal mismatch distributions would be expected if recent range expansions following a population bottleneck had occurred (Ding, 2011; Fu, 1997; Rogers and Harpending, 1992; Slatkin and Hudson 1991; Tajima, 1989). Additionally, the possibility of a recent population bottleneck in microsatellite data was assessed using BOTTLENECK v. 1.2.02 (Cornuet and Luikart, 1996). The significant presence of an excess of observed heterozygotes was tested using the Wilcoxon signed rank test. Heterozygosity excess was tested under an infinite alleles model (IAM), a step-wise mutation model (SMM), and the two-phase model (TPM). For TPM we set a variance of 30 and a stepwise mutation probability of 70%. The allele mode shift test (distortion from L-shape allele distribution) was also tested using the same software.

3. Results

3.1 Mitochondrial and microsatellite genetic diversity

In *A. purpuratus*, the complete 16S rRNA gene sequence (GenBank accession number: HQ677600) was 1317 bp in length, and the A+T content was 58.47%. Mitochondrial intrapopulation diversity indices are shown in Table 1. A 530 bp size product of the 16S rRNA gene was successfully sequenced in 68 individuals from three

Peruvian localities. In total, 19 sites were variable and 18 different haplotypes were found among all samples. High values of h (0.6947-0.8360) were found in all localities, SEB population showed the highest h (0.8360). Haplotypes H01 and H13 were the common ones and were found in all populations, being haplotype H01 the most dominant (44.12%). Six haplotypes (H2, H3, H4, H8, H9 and H12) were shared by two populations and 10 haplotypes were found only in one population. All populations showed at least one unique haplotype. Similar π values were in all populations, ranging from 0.002092 (SAB population) to 0.002294 (INB population).

The calculated data of microsatellite genetic diversity for each population are shown in Table 2. A total of 112 alleles were detected across nine microsatellite loci ranging from two at the locus *APPE23* in INB population to 15 at the locus *APPE9* in SEB population. The highest and lowest average number of alleles was found in SEB and INB populations, respectively. The allelic richness (R_s) ranged from 1.99 (locus *APPE23*) to 11.93 (locus *APPE9*) both in INB population. On average, SAB population showed the highest R_s value (7.66). The H_O and H_E values ranged from 0.1333 to 1.0 and from 0.0684 to 0.8921, respectively.

After adjusting for multiple comparisons, significant departures from HWE were observed in three loci (*APPE9*, *APPE11* and *APPE20*). Analyses using MICRO-CHECKER suggested the presence of null alleles at these three loci. Locus *APPE20* showed significant deviation across the three populations analyzed and was therefore omitted from further analyses. Two pairs of loci were in linkage disequilibrium (*APPE11-APPE17* and *APPE13-APPE22*) after Bonferroni correction. Since gametic disequilibrium creates pseudo-replication for analysis in which loci are assumed to act as

independent markers, one locus of the pair should be excluded if significant disequilibrium is found between loci (Selkoe and Toonen, 2006). Consequently, loci *APPE11* and *APPE22* were excluded from further analyses.

3.2 Population genetic structure

AMOVA for mitochondrial haplotype data showed very similar Φ -statistic results when populations were grouped as a single gene pool and when were divided into two groups (Table 3). None of the Φ -statistics were significant. Single gene pool AMOVA indicated that 99.49% of the variation occurred within populations and only 0.51% of the overall variance was due to differences among populations. This result was supported by pairwise Φ_{ST} values, which revealed no significant genetic structure among the three locations (Table 4), pairwise Φ_{ST} values among populations ranged from -0.1241 (between SEB and INB) to 0.02961 (between SAB and INB).

A minimum spanning network based on nucleotide divergences among haplotypes was constructed using ARLEQUIN software (Fig. 2), resulting in a star-like shape network, which is commonly observed in marine organisms, indicating that most haplotypes were closely related with a centrally located ancestral haplotype (H1).

Contrastingly to mitochondrial analyses, AMOVA for microsatellite loci showed a small (2.86%) but highly significant ($P=0.0001$) genetic variation among the three localities (Table 5). However, most of the genetic variance (97.69%) was explained by differences within individuals. The pairwise F_{ST} estimates among populations varied from 0.01948 to 0.07718, this analysis indicated that INB population was responsible for

most of the difference (Table 6).

The Bayesian STRUCTURE clustering approach using multilocus microsatellite genotypes failed to detect population structure: a maximum posterior probability was given for $K=1$ ($\ln P[K/X] = -1075.2$; data not shown). Additionally, the symmetric proportion of individuals assigned to putative clusters indicated little or no genetic structure.

3.3 Historical demography

Overall results from neutrality tests Tajima's D and Fu's F_S showed significant negative values indicating past population expansion (Table 7). Fu's F_S test resulted in significant negative values for all populations whereas Tajima's D test showed negative values for all populations but only SEB differs significantly from neutrality. The mismatch distribution including all samples was consistent with models of population expansion, displaying a unimodal distribution (Fig. 3). Test of the observed Hri statistics failed to reject the null hypothesis of recent population expansion, confirming the mismatch distribution results.

However, BOTTLENECK analysis using microsatellite data showed no evidence of recent population bottleneck. No significant heterozygosity excess was detected in any locality under all three mutational models (Table 8). Moreover, mode-shift indicator showed a normal "L" shaped distribution in all populations, thus indicating the absence of bottleneck.

4. Discussion

Marine species with a long planktonic larvae phase could be expected to show lower level of genetic differentiation than terrestrial or freshwater species, typically due to high degree of gene flow in marine environments, large population sizes, high fecundity and larval drift during planktonic stage (Cano et al., 2008; Ni et al., 2011). The conception that marine environments do not have “hard” barriers which tend to promote the dispersion of many species during eggs or larval stages, agreed with many genetic studies that indicated a lack of genetic structure even over large geographic distances (Ward et al., 1994). This could be the case of marine bivalves from the family Pectinidae in which, regardless of species, pelagic life starts with the release of gametes, followed by fertilization and embryonic and larval stages until metamorphosis and recruitment to the benthic community (Le Pennec et al., 2003). However, significant genetic structure has been observed in pectinid populations by using microsatellite markers (e.g. *M. yessoensis*, An et al., 2009; *A. irradians*, Hemond and Wilbur, 2011; *C. farreri*, Zhan et al., 2009a).

Intraspecific diversity of 16S rRNA gene indicated that SEB population has the richest variation among all populations, which may be explained by “explosive” biomass proliferation in this bay probably due to seed introduction from other localities (Mendo et al., 2008). The other explanation is that SEB sample size ($n=28$) was relatively larger than those of the other two populations ($n=20$, each).

Moderate to high level of microsatellite heterozygosity was detected in all populations. High genetic diversity is a common characteristic in marine bivalves (Zhan et al., 2009a) and has been previously reported in *A. purpuratus* (Moragat et al., 2001;

von Brand and Kijima, 1990). Mean microsatellite H_E was higher than H_O in all Peruvian scallop populations and three loci showed significant deviation from HWE. In bivalves, heterozygote deficiencies and departures from HWE in microsatellite analysis are common and mainly attributed to inbreeding, genetic patchiness (Walhund effect), or null alleles (Lemer et al., 2011).

In *A. purpuratus*, the 16S rRNA gene showed a similar level of variation when compared with other bivalve studies. In a 592 bp partial 16S rRNA gene fragment, Kong et al. (2003) reported 31 polymorphic sites and 23 haplotypes in 47 individuals of *C. farreri*. In a 534 bp fragment of the same mitochondrial gene Mahidol et al. (2007) observed 27 polymorphic sites and 16 unique haplotypes among 174 individuals of *Amusium pleuronectes*. In a 468 bp fragment of the 16S rRNA gene determined in 221 individuals of *C. nobilis*, Yuan et al. (2009) reported 24 polymorphic sites and 27 haplotypes. However, lower levels of polymorphism for the 16S rRNA gene have been reported in the bivalve *Pinna nobilis*, in a 489 bp partial fragment analyzed in 25 individuals only two different haplotypes were detected (Katsares et al., 2008).

Overall, high mean h (0.77) and low levels of mean π (0.0022) were shown in this study. These results may be related to expansion after a period of low effective population size, because rapid population growth enhances the retention of new mutations (Avice et al., 1984; Stamatis et al., 2004). Indeed, the demographic expansion following a population bottleneck scenario is supported by significantly negative Tajima's D and Fu's F_S values, unimodal mismatch distribution, and a star-shaped haplotype network found in this study. In Peru, scallops were harvested until depletion of the stocks, especially when favorable environmental conditions (i.e. El Niño events)

increased scallop biomass in certain bays (Wolff et al., 2007; Mendo et al., 2008). Such a situation could cause population bottleneck effects in Peruvian scallop stocks. Genetic signatures of bottleneck and demographic expansion have been detected in other commercially exploited scallops (Bert et al., 2011; Gaffney et al., 2010). However, microsatellite heterozygosity-based bottleneck test did not show signals of population bottleneck. A possible reason for the lack of population decline signal could be attributed to the rapid demographic recovery rate of this species: marine bivalves are known for their high fecundity (Zhan et al., 2009b), in *A. purpuratus* spawning occurs throughout the year (Wolff, 1988), and it is a fast-growing species (von Brand et al., 2006). Rapid population expansion is expected to promote the introduction of new alleles (Slatkin and Hudson, 1991). However these new alleles are at a low frequency creating an excess of allelic diversity above what would be predicted from a nongrowing population at mutation-drift equilibrium (Lawler, 2008), which will tend to erode pre-existing signal of heterozygosity excess (Cornuet and Luikart, 1996). In this study Tajima's D test showed negative values for all populations, however only SEB population resulted in statistically significant data. On the other hand, Fu's F_S test resulted in significant negative values for all populations. It has been shown that Tajima's D test is less powerful than Fu's F_S (Ramos-Onsins and Rozas, 2002), which could explain the different significant values found in some populations.

Despite the fact that some degree of genetic variation was observed in mitochondrial analyses of *A. purpuratus* populations, no statistically significant genetic structure was detected among Peruvian scallop localities. Lack of genetic structure indicates that gene flow is occurring among populations, which may be explained by the

existence of planktonic stage in shellfish larvae. Developing larvae spend a variable period of time (averagely two weeks in *A. purpuratus*) as part of plankton, which can be passively drifted by water currents, often over considerable distances (Gharbi et al., 2010). Translocation of scallop spats from one locality to another for aquaculture activities could be another possible factor to explain the lack of significant genetic structure in Peruvian scallop populations. Especially after El Niño events, to increase scallop production, seed from wild stocks had been introduced into other localities (Mendo et al., 2008). Overall, low to moderate F_{ST} values were obtained in this study. Similar low F_{ST} values have been found in other population studies of scallop species (Gaffney et al., 2010; Wilding et al., 1997). However, there are an increasing number of publications about sessile marine invertebrate that show population subdivision, despite planktonic larvae dispersal potential, in which F_{ST} values ranged from 0.007 to 0.28 (Whitaker, 2004; and references therein). Bradbury et al. (2008) surveyed published F_{ST} values for 246 marine species (83 invertebrate and 163 fish species), the values were primarily between 0 and 0.05, although a significant portion ranged as high as 0.4, indicating restricted gene flow among populations.

Lack of genetic structure

among Peruvian scallop samples was also detected with STRUCTURE software ($K=1$). On the other hand, AMOVA analysis based on microsatellite data indicated low but highly significant ($P=0.0001$) levels of genetic differentiation among Peruvian scallop localities. Previous studies have discussed the sensitivity of Bayesian methods when dealing with weak genetic structuring, and it has been demonstrated that their performance depend on the levels of population differentiation (Franchini et al., 2012; Latch et al., 2006). In this study, STRUCTURE results may be biased by the low levels

of genetic differentiation among Peruvian scallop populations. However, care must be taken when interpreting the results, especially considering the sample size analyzed in the present study. Small sample sizes can result in biased estimates of the allele frequencies of each subpopulation, particularly when dealing with highly polymorphic markers (e.g. microsatellites), which may affect the estimation of F_{ST} 's (Kitada et al., 2007). Pairwise F_{ST} values showed that most of the significant genetic structure was attributed to INB population. A possible explanation for the differences found in INB population could be related to its geographic position: this bay is located in Paracas National Reserve and apparently has not been yet subjected to seed transplantation (Mendo et al., 2008). Geographic distances might also influence the gene flow among populations, INB population is located 1000 and 600 km apart from SEB and SAB populations, respectively. It has been suggested that larvae carried by marine currents are subjected to a high mortality (Cameron, 1986). When larvae are passively drifted over a long distance, they must overcome environmental differences to successfully become mature (Cho et al., 2007). The water circulation in the Peruvian marine ecosystem is characterized by a northward flow called Humboldt Current, this extends from central Chile (40°S) to northern Peru (4-5°S) (Tarazona et al., 2003). This current could promote gene flow among *A. purpuratus* populations. However, natural beds of *A. purpuratus* occur specially in sheltered inshore bays with limited water exchange (Illanes, 1990). This supports the result by Moragat et al. (2001), in which significant differentiation was found between two wild populations of *A. purpuratus* from Chile based on allozymes and morphological features. Whereas, Independencia Bay is subjected to physical variability influenced by strong currents and the presence of anticyclonic eddies have been observed

in this bay (Vélez et al., 2005). Anticyclonic eddy structures act as strong retention zones for marine larvae by recirculating or accumulating them in regions where secondary convergent flows occur (Landeira et al., 2010; Sponaugle et al., 2002). *A. purpuratus* larval retention due to the presence of current gyres has been previously reported in a Chilean bay (Avendaño et al., 2007). Furthermore, it has been shown that marine larvae can avoid being passively drifting by currents by modifying their behavior and regulating their vertical position in the water column (Brooke and Young, 2005). Thus, the potential for gene flow through larval drift from INB population may be limited. Two main factors may explain the discrepancies between the mitochondrial and nuclear population structure estimates. First, microsatellite has higher mutation rates than mitochondrial DNA (Frankham et al., 2002) which could lead to greater population differentiation (Sellas et al., 2005). A second reason is that there are different intensities of selection on each marker (Johnson et al., 2003). The discrepancies between microsatellite and mitochondrial DNA markers highlight the importance of using more than one type of marker when making inferences about the genetic structure of populations (Sellas et al., 2005).

Further studies with larger sample sizes in each population using more microsatellite markers are required. More molecular data will be useful to assist with sustainable fisheries management and conservation of this resource. Overfishing has caused serious population depletion of different scallop species such as *C. islandica* (Garcia, 2006), *A. irradians* (Hemond and Wilbur, 2011), *Pecten spp.* (Saavedra and Peña, 2004), *A. purpuratus* (Stotz and Mendo, 2001). Proper knowledge of the stock structure is necessary to prevent overfishing, preserve genetic diversity and to ensure sustainable exploitation of the stocks (Jónsdóttir et al., 2006). In this study, INB

population was weakly differentiated from other Peruvian populations. If Independencia Bay is confirmed to remain as a genetically uncontaminated population, it should be considered as a source of “fresh” alleles for future aquaculture and restocking programs.

Acknowledgements

Part of this work was presented on the 18th International Pectinid Workshop, Qingdao-China, 2011. We thank Ruben Alfaro (National University of Trujillo, Peru) and Elmer Ramos (National University of San Marcos, Peru) for providing *A. purpuratus* samples and Ryota Yokoyama, John Bower, Hideharu Tsukagoshi and Hokuto Shirakawa (Hokkaido University) for their helpful advice. Two anonymous reviewers greatly helped to improve the quality of this paper.

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Figure captions

Figure 1. Peruvian sampling locations of *A. purpuratus*.

Figure 2. A minimum spanning network based on the mitochondrial 16S rRNA gene sequences from *A. purpuratus*. Each haplotype is represented by a circle with the area proportional to its relative abundance and colour indicating geographical distribution. Lines connecting haplotypes and black dots indicate a single nucleotide difference.

Figure 3. Mismatch distributions of mitochondrial 16S rRNA gene haplotypes for all pairwise combinations (pooled sample, $n=68$) of the Peruvian scallops.

Table 1. Distribution of 18 haplotypes of the mitochondrial 16S rRNA gene, number of polymorphic sites, number of haplotypes, haplotype diversity, and nucleotide diversity in three *A. purpuratus* samples.

Variable nucleotide site																			Haplotype frequency			
Haplo type	5	1	2	4	5	5	5	6	8	1	1	1	2	2	2	2	4	5	5	SEB (<i>n</i> =28)	SAB (<i>n</i> =20)	INB (<i>n</i> =20)
H1	G	A	C	T	T	A	T	G	T	A	G	A	T	C	A	A	C	G	C	0.3929	0.4000	0.5500
H2	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	0.1071		0.0500
H3	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	0.1071	0.3000	
H4	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	.	.	0.0357	0.0500	
H5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	0.0714		
H6	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0357		
H7	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	0.0357		
H8	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	A	.	0.0357	0.0500	
H9	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	A	T	0.0357	0.0500	
H10	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	0.0357		
H11	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0357		
H12	.	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.	T	A	.	0.0357		0.0500
H13	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	G	.	A	.	0.0357	0.1000	0.1500
H14	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.		0.5000	
H15	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.			0.0500
H16	.	.	.	.	.	G	.	.	.	G	.	.	.	.	.	.	.	.	.			0.0500
H17	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.			0.0500
H18	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			0.0500
Number of polymorphic sites																				13	7	10
Number of haplotypes																				13	7	8
Haplotype diversity (<i>h</i>)																				0.8360	0.7684	0.6947
Nucleotide diversity (π)																				0.00217	0.002092	0.00229
																				1		4

n, number of analyzed individuals in each population.

Table 2. Genetic variation at nine microsatellite loci for three *A. purpuratus* populations.

Popula- tion	Locus									
	<i>APPE9</i>	<i>APPE11</i>	<i>APPE13</i>	<i>APPE17</i>	<i>APPE18</i>	<i>APPE20</i>	<i>APPE22</i>	<i>APPE23</i>	<i>APPE25.1</i>	All loci
SEB (<i>n</i> =29)										
<i>A</i>	15	13	8	14	7	10	8	3	4	9.11/82
<i>R_s</i>	11.74	10.88	6.34	10.99	4.96	8.33	6.38	2.03	3.77	7.27
S (bp)	254-296	179-239	180-327	368-428	380-624	301-424	276-363	177-186	145-154	-
<i>H_O</i>	0.6897	0.6071	0.5172	0.8276	0.6897	0.2500	0.7308	0.0689	0.6207	0.5558
<i>H_E</i>	0.8814	0.8675	0.6842	0.8832	0.6080	0.6416	0.6976	0.0684	0.5463	0.6531
<i>P</i>	0.0000	0.0000	0.0168	0.0326	0.1202	0.0000	0.4019	1.000	0.7923	-
SAB (<i>n</i> =20)										
<i>A</i>	11	11	8	11	4	12	10	3	4	8.22/74
<i>R_s</i>	10.16	10.27	7.76	10.08	3.67	10.79	9.61	2.69	3.94	7.66
S (bp)	257-293	179-230	238-321	368-431	457-544	327-433	261-357	177-186	148-157	-
<i>H_O</i>	0.7222	0.8421	0.5625	1.000	0.5000	0.2778	0.4706	0.1500	0.6250	0.5722
<i>H_E</i>	0.8921	0.8805	0.6673	0.8791	0.5429	0.8254	0.8057	0.1449	0.5020	0.6822
<i>P</i>	0.0847	0.1031	0.1705	0.4990	1.000	0.0000	0.0061	1.000	0.8184	-
INB (<i>n</i> =20)										
<i>A</i>	13	10	9	10	4	8	7	2	4	7.44/67
<i>R_s</i>	11.93	10.0	7.75	8.91	3.79	8.00	6.44	1.99	3.75	6.95
S (bp)	254-296	177-239	180-313	368-455	380-547	327-563	273-357	177-183	148-160	-
<i>H_O</i>	1.000	1.000	0.7000	0.9474	0.6316	0.1333	0.7000	0.2000	0.5500	0.6514
<i>H_E</i>	0.8890	0.8575	0.7474	0.8393	0.5846	0.8414	0.7231	0.1846	0.5679	0.6928
<i>P</i>	0.8479	0.0674	0.1449	0.4243	0.0034	0.0000	0.5316	1.000	0.3574	-

n, sample size; *A*, allele number; *R_s*, allelic richness; *S*, allele size range (bp); *H_O*, observed heterozygosity; *H_E*, expected heterozygosity; *P*, exact *P* value for Hardy-Weinberg equilibrium test; in which values in bold type are significant probability estimates after correction for multiple tests

Table 3. Analysis of molecular variance (AMOVA) for mitochondrial 16S rRNA gene haplotypes of *A. purpuratus* from three locations.

Structure tested	Observed partition	% total	Φ statistics	P
	Variance			
1. One gene pool (SEB, SAB and INB)				
Among populations	0.00440	0.51	Φ_{ST} =0.0051	0.321
Within populations	0.85637	99.49		
2. Two groups (SEB and SAB)(INB)				
Among groups	0.00186	0.22	Φ_{CT} =0.0022	0.664
Within groups	0.00322	0.37	Φ_{SC} =0.0038	0.313
Within populations	0.85637	99.41	Φ_{ST} =0.0059	0.314

Table 4. Population pairwise Φ_{ST} values (above diagonal) and statistical P values (below diagonal) in three populations of *A. purpuratus* based on partial 16S gene haplotypes.

	SEB	SAB	INB
SEB	-	0.00486	-0.1241
SAB	0.31314	-	0.02961
INB	0.65132	0.17642	-

Table 5. Analysis of molecular variance (AMOVA) in three *A. purpuratus* samples using six microsatellite loci.

Source of variation	d.f.	Variance components	Percentage of variation
Among populations	2	0.05194	2.86*
Among individuals within populations	57	-0.01004	-0.55
Within individuals	60	1.77500	97.69
Total	119	1.81689	

*Significant at $P < 0.05$, 10100 permutations.

Table 6. Population pairwise F_{ST} values (above diagonal) and statistical P values (below diagonal) in three populations of *A. purpuratus* based on six microsatellite loci.

	SEB	SAB	INB
SEB	-	0.01948	0.05370
SAB	0.02995	-	0.07718
INB	0.0001*	0.0001*	-

*Significant at $P < 0.05$ after Bonferroni correction.

Table 7. Demographic estimates from mitochondrial 16S partial gene for Peruvian scallop populations.

Locality	Fu F_s (P)	Tajima D (P)	SSD (P)	Raggedness index (P)
Overall	-11.2900 (0.0001)*	-1.71518 (0.01730)*	0.1018	0.0415 (0.98790)
SEB	-8.4200 (0.0001)*	-1.62619 (0.03260)*	0.00517 (0.41600)	0.06820 (0.37690)
SAB	-1.8924 (0.0329)*	-0.61293 (0.27900)	0.01718 (0.39170)	0.08825 (0.45710)
INB	-2.5570 (0.0460)*	-1.25075 (0.09880)	0.01309 (0.68530)	0.04410 (0.87190)

*Significant at $P < 0.05$

Table 8. Summary of the results for BOTTLENECK analyses for microsatellite data of six polymorphic loci using Wilcoxon signed rank test.

Population	Mutational model	Heterozygosity excess	Mode shift
SEB	IAM	0.719	NS
	TPM	1.000	NS
	SMM	1.000	NS
SAB	IAM	0.578	NS
	TPM	0.945	NS
	SMM	0.977	NS
INB	IAM	0.055	NS
	TPM	0.945	NS
	SMM	0.977	NS

NS, no significant

Supplementary material

Table 1. Primers used to develop the mitochondrial 16S rRNA gene in *A. purpuratus*.

Primer Name	Sequence (5'-3')	Source
16S R	CCGRTYTGAAC TCAGCTCACG	Puslednik and Serb (2008)
16S arl	CGCCTGTTTAACAAAAACAT	Palumbi et al. (1991)
ND1E-forward	CGGCTTCGCCATGATCttyatygcnga	This study
CO1AB-reverse	GGTGCTGGGCAGCcayatncngg	This study
ND1EAA	GATAAGGTGTTTTGGCATC	This study
16SAA	GGTCCCACCTAGAAGCTAATG	This study
16SBB	GGTAAAGCGTGCTAAGGTA	This study
16SCC	GCGTAATCCGTCTTGACAGT	This study

Table 2. Microsatellites loci used to analyze the *A. purpuratus* population structure in this study.

Locus	Motif	Fluorescent label	T_A (°C)	Reference
APPE9	Trinucleotide	VIC	56	Marín et al. (2012)
APPE11	Trinucleotide	FAM	56	Marín et al. (2012)
APPE13	Trinucleotide	NED	56	Marín et al. (2012)
APPE17	Trinucleotide	VIC	56	Marín et al. (2012)
APPE18	Trinucleotide	FAM	56	Marín et al. (2012)
APPE20	Trinucleotide	NED	56	Marín et al. (2012)
APPE22	Trinucleotide	PET	56	Marín et al. (2012)
APPE23	Trinucleotide	FAM	60	Marín et al. (2012)
APPE25.1	Trinucleotide	PET	60	Marín et al. (2012)