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<td>Author(s)</td>
<td>Marín, Alan; Fujimoto, Takafumi; Arai, Katsutoshi</td>
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<td>Citation</td>
<td>Marine Genomics, 9: 1-8</td>
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<tr>
<td>Issue Date</td>
<td>2013-03</td>
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<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/52046">http://hdl.handle.net/2115/52046</a></td>
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Genetic structure of the Peruvian scallop *Argopecten purpuratus* inferred from mitochondrial and nuclear DNA 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; Villella et al., 1998). It is well known that most marine species have a planktonic larval stage, which may result in a high gene flow
between populations due to passive larval dispersal by marine currents (Cho et al., 2007; Katsares et al., 2008). Most of such marine species are thought to have little genetic structure (Yuan et al., 2009). However, extrinsic factors including marine currents and gyres, hydrographical barriers to dispersal, bays and islands, and even anthropogenic activities can affect the population structure of several marine bivalves (Ni et al., 2011; Zhan et al., 2009a). Significant genetic differentiation over small geographic scales has been revealed in clams, mussels, and scallops (Luttikhuizen et al., 2003; Ni et al., 2011; Ridgway, 2001; Zhan et al., 2009a). A. purpuratus is a continuous spawner species with spawning peaks during late summer and autumn (Wolff, 1988) and its larval stage has been reported to last between 16 to 25 days under hatchery conditions (von Brand et al., 2006). Thus, due to its high dispersal potential, this species is expected to have little or no genetic structure. Nevertheless, in spite of its great commercial and ecological values, so far little is known about the genetic variation and population structure of A. purpuratus stocks. To date, population genetic studies have not been conducted in A. purpuratus except for classic approach using allozymes as genetic markers. For example, Galleguillos and Troncoso (1991) found no genetic differences among Chilean populations using allozyme electrophoresis. However, Moragat et al. (2001), using allozymes and morphological characters detected statistically significant differences between two A. purpuratus populations from northern Chile. These contrasting results based both on allozyme analyses could be attributed to the different number and polymorphic loci found and analyzed in each study and also to the oceanographic geographical features of the surveyed bays. Microsatellite and mitochondrial markers have been used to determine the
population structure in Pectinidae species including *Patinoptecten caurinus* (Gaffney et al., 2010), *A. iradians* (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'-CGGCTTCGCCATGATCttyatygcnga-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 16Sarl (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 16Sarl-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 $\Phi_{ST}$ 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 MICROCHECKER 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 100000 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 $\pi$ 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 $H_0$ 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 $\Phi$-statistic results
when populations were grouped as a single gene pool and when were divided into two
groups (Table 3). None of the $\Phi$-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 $\Phi_{ST}$ values, which revealed no significant genetic structure among the three
locations (Table 4), pairwise $\Phi_{ST}$ 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;
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 $\pi$ (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 (Avise 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*<sub>S</sub> 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*<sub>S</sub> (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.

References


Jónsdóttir, I.G., Campana, S.E., Marteinsdottir, G., 2006 Stock structure of Icelandic cod *Gadus morhua* L. based on otolith chemistry. J. Fish Biol. 69, 136-150.


Saavedra, C., Peña, J.B., 2004. Phylogenetic relationship of commercial European and
Australasian king scallops (Pecten spp.) based on partial 16S ribosomal RNA gene
sequences. Aquaculture 235, 153-166.

using and evaluating microsatellite markers. Ecol. Lett. 9, 615-629.

Sellas, A.B., Wells, R.S., Rosel, P.E., 2005. Mitochondrial and nuclear DNA analyses
reveal fine scale geographic structure in bottlenose dolphins (Tursiops truncatus) in
the Gulf of Mexico. Conserv. Genet. 6, 715-728.

in stable and exponentially growing populations. Genetics 129, 555-562.

Stotz, W.B., Mendo, J., 2001. Pesquerías, repoblamiento y manejo de bancos naturales de
pectínidos en Iberoamérica: su interacción con la acuicultura. A.N. Maeda-
Martinez (Ed.), Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura, Limusa,
Mexico, pp. 357-374.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA

of Marine Biodiversity Research in Peru. Gayana (Concepc.) 67, 206-231.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the
sensitivity of progressive multiple sequence alignment through sequence weighting,
position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673-
4680.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-
CHECKER: software for identifying and correcting genotyping errors in microsatellite

assemblages in Independencia Bay, Pisco, Peru: temporal and spatial relationships.


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.
Observed

Constant population model

Growth-declined population model
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.

<table>
<thead>
<tr>
<th>Variable nucleotide site</th>
<th>Haplotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEB (n=28)</td>
</tr>
<tr>
<td>H1</td>
<td>0.3929</td>
</tr>
<tr>
<td>H2</td>
<td>0.1071</td>
</tr>
<tr>
<td>H3</td>
<td>0.0714</td>
</tr>
<tr>
<td>H4</td>
<td>0.0357</td>
</tr>
<tr>
<td>H5</td>
<td>0.0357</td>
</tr>
<tr>
<td>H6</td>
<td>0.0357</td>
</tr>
<tr>
<td>H7</td>
<td>0.0357</td>
</tr>
<tr>
<td>H8</td>
<td>0.0357</td>
</tr>
<tr>
<td>H9</td>
<td>0.0357</td>
</tr>
<tr>
<td>H10</td>
<td>0.0357</td>
</tr>
<tr>
<td>H11</td>
<td>0.0357</td>
</tr>
<tr>
<td>H12</td>
<td>0.0357</td>
</tr>
<tr>
<td>H13</td>
<td>0.0357</td>
</tr>
<tr>
<td>H14</td>
<td>0.0500</td>
</tr>
<tr>
<td>H15</td>
<td>0.0500</td>
</tr>
<tr>
<td>H16</td>
<td>0.0500</td>
</tr>
<tr>
<td>H17</td>
<td>0.0500</td>
</tr>
<tr>
<td>H18</td>
<td>0.0500</td>
</tr>
<tr>
<td>Number of polymorphic sites</td>
<td>13</td>
</tr>
<tr>
<td>Number of haplotypes</td>
<td>13</td>
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<td>Haplotype diversity (h)</td>
<td>0.8360</td>
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<tr>
<td>Nucleotide diversity ((\pi))</td>
<td>0.00217</td>
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</tbody>
</table>

\(n\), number of analyzed individuals in each population.
<table>
<thead>
<tr>
<th>Locus</th>
<th>APPE9</th>
<th>APPE11</th>
<th>APPE13</th>
<th>APPE17</th>
<th>APPE18</th>
<th>APPE20</th>
<th>APPE22</th>
<th>APPE23</th>
<th>APPE25.1</th>
<th>All loci</th>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>(n=29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>15</td>
<td>13</td>
<td>8</td>
<td>14</td>
<td>7</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>9.11/82</td>
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<tr>
<td>Rs</td>
<td>11.74</td>
<td>10.88</td>
<td>6.34</td>
<td>10.99</td>
<td>4.96</td>
<td>8.33</td>
<td>6.38</td>
<td>2.03</td>
<td>3.77</td>
<td>7.27</td>
</tr>
<tr>
<td>S (bp)</td>
<td>254-296</td>
<td>179-239</td>
<td>180-327</td>
<td>368-428</td>
<td>380-624</td>
<td>301-424</td>
<td>276-363</td>
<td>177-186</td>
<td>145-154</td>
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<td>H₀</td>
<td>0.6897</td>
<td>0.6071</td>
<td>0.5172</td>
<td>0.6897</td>
<td>0.2500</td>
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<td>Hₑ</td>
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<td>0.8675</td>
<td>0.6842</td>
<td>0.8332</td>
<td>0.6080</td>
<td>0.6416</td>
<td>0.6976</td>
<td>0.0684</td>
<td>0.5463</td>
<td>0.6531</td>
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<td>0.0000</td>
<td>0.0000</td>
<td>0.0168</td>
<td>0.0326</td>
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<td>0.4019</td>
<td>1.000</td>
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<td>A</td>
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<td>11</td>
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<td>12</td>
<td>10</td>
<td>3</td>
<td>4</td>
<td>8.22/74</td>
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<tr>
<td>Rs</td>
<td>10.16</td>
<td>10.27</td>
<td>7.76</td>
<td>10.08</td>
<td>3.67</td>
<td>10.79</td>
<td>9.61</td>
<td>2.69</td>
<td>3.94</td>
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<tr>
<td>S (bp)</td>
<td>257-293</td>
<td>179-230</td>
<td>238-321</td>
<td>368-431</td>
<td>457-544</td>
<td>327-433</td>
<td>261-357</td>
<td>177-186</td>
<td>148-157</td>
<td>-</td>
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<tr>
<td>H₀</td>
<td>0.7222</td>
<td>0.8421</td>
<td>0.5625</td>
<td>1.000</td>
<td>0.5000</td>
<td>0.2778</td>
<td>0.4706</td>
<td>0.1500</td>
<td>0.6250</td>
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<tr>
<td>Hₑ</td>
<td>0.8921</td>
<td>0.8805</td>
<td>0.6673</td>
<td>0.8791</td>
<td>0.5429</td>
<td>0.8254</td>
<td>0.8057</td>
<td>0.1449</td>
<td>0.5020</td>
<td>0.6822</td>
</tr>
<tr>
<td>P</td>
<td>0.0847</td>
<td>0.1031</td>
<td>0.1705</td>
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<td>1.000</td>
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<td>0.0061</td>
<td>1.000</td>
<td>0.8184</td>
<td>-</td>
</tr>
<tr>
<td>INB</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>13</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>7.44/67</td>
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<tr>
<td>Rs</td>
<td>11.93</td>
<td>10.00</td>
<td>7.75</td>
<td>8.91</td>
<td>3.79</td>
<td>8.00</td>
<td>6.44</td>
<td>1.99</td>
<td>3.75</td>
<td>6.95</td>
</tr>
<tr>
<td>S (bp)</td>
<td>254-296</td>
<td>177-239</td>
<td>180-313</td>
<td>368-455</td>
<td>380-547</td>
<td>327-563</td>
<td>273-357</td>
<td>177-183</td>
<td>148-160</td>
<td>-</td>
</tr>
<tr>
<td>H₀</td>
<td>1.000</td>
<td>1.000</td>
<td>0.7000</td>
<td>0.9474</td>
<td>0.6316</td>
<td>0.1333</td>
<td>0.7000</td>
<td>0.2000</td>
<td>0.5500</td>
<td>0.6514</td>
</tr>
<tr>
<td>Hₑ</td>
<td>0.8890</td>
<td>0.8575</td>
<td>0.7474</td>
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<td>0.5846</td>
<td>0.8414</td>
<td>0.7231</td>
<td>0.1846</td>
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<td>0.6928</td>
</tr>
<tr>
<td>P</td>
<td>0.8479</td>
<td>0.0674</td>
<td>0.1449</td>
<td>0.4243</td>
<td>0.0034</td>
<td>0.0000</td>
<td>0.5316</td>
<td>1.000</td>
<td>0.3574</td>
<td>-</td>
</tr>
</tbody>
</table>

n, sample size; A, allele number; Rs, allelic richness; S, allele size range (bp); H₀, observed heterozygosity; Hₑ, 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.

<table>
<thead>
<tr>
<th>Structure tested</th>
<th>Observed partition</th>
<th>Variance</th>
<th>% total</th>
<th>$\Phi$ statistics</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. One gene pool (SEB, SAB and INB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>0.00440</td>
<td>0.51</td>
<td>$\Phi_{ST}=0.0051$</td>
<td>0.321</td>
<td></td>
</tr>
<tr>
<td>Within populations</td>
<td>0.85637</td>
<td>99.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Two groups (SEB and SAB)(INB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among groups</td>
<td>0.00186</td>
<td>0.22</td>
<td>$\Phi_{CT}=0.0022$</td>
<td>0.664</td>
<td></td>
</tr>
<tr>
<td>Within groups</td>
<td>0.00322</td>
<td>0.37</td>
<td>$\Phi_{SC}=0.0038$</td>
<td>0.313</td>
<td></td>
</tr>
<tr>
<td>Within populations</td>
<td>0.85637</td>
<td>99.41</td>
<td>$\Phi_{ST}=0.0059$</td>
<td>0.314</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Population pairwise $\Phi_{ST}$ values (above diagonal) and statistical $P$ values (below diagonal) in three populations of *A. purpuratus* based on partial 16S gene haplotypes.

<table>
<thead>
<tr>
<th></th>
<th>SEB</th>
<th>SAB</th>
<th>INB</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEB</td>
<td>-</td>
<td>0.00486</td>
<td>-0.1241</td>
</tr>
<tr>
<td>SAB</td>
<td>0.31314</td>
<td>-</td>
<td>0.02961</td>
</tr>
<tr>
<td>INB</td>
<td>0.65132</td>
<td>0.17642</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5. Analysis of molecular variance (AMOVA) in three *A. purpuratus* samples using six microsatellite loci.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Variance components</th>
<th>Percentage of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>2</td>
<td>0.05194</td>
<td>2.86*</td>
</tr>
<tr>
<td>Among individuals within populations</td>
<td>57</td>
<td>-0.01004</td>
<td>-0.55</td>
</tr>
<tr>
<td>Within individuals</td>
<td>60</td>
<td>1.77500</td>
<td>97.69</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>1.81689</td>
<td></td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th></th>
<th>SEB</th>
<th>SAB</th>
<th>INB</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEB</td>
<td></td>
<td>0.01948</td>
<td>0.05370</td>
</tr>
<tr>
<td>SAB</td>
<td>0.02995</td>
<td></td>
<td>0.07718</td>
</tr>
<tr>
<td>INB</td>
<td>0.0001*</td>
<td>0.0001*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at $P<0.05$ after Bonferroni correction.
Table 7. Demographic estimates from mitochondrial 16S partial gene for Peruvian scallop populations.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Fu $F_i (P)$</th>
<th>Tajima $D (P)$</th>
<th>SSD ($P$)</th>
<th>Raggedness index ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>-11.2900 (0.0001)*</td>
<td>-1.71518 (0.01730)*</td>
<td>0.1018</td>
<td>0.0415 (0.98790)</td>
</tr>
<tr>
<td>SEB</td>
<td>-8.4200 (0.0001)*</td>
<td>-1.62619 (0.03260)*</td>
<td>0.00517 (0.41600)</td>
<td>0.06820 (0.37690)</td>
</tr>
<tr>
<td>SAB</td>
<td>-1.8924 (0.0329)*</td>
<td>-0.61293 (0.27900)</td>
<td>0.01718 (0.39170)</td>
<td>0.08825 (0.45710)</td>
</tr>
<tr>
<td>INB</td>
<td>-2.5570 (0.0460)*</td>
<td>-1.25075 (0.09880)</td>
<td>0.01309 (0.68530)</td>
<td>0.04410 (0.87190)</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Population</th>
<th>Mutational model</th>
<th>Heterozygosity excess</th>
<th>Mode shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEB</td>
<td>IAM</td>
<td>0.719</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TPM</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>SMM</td>
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<td>NS</td>
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<tr>
<td>SAB</td>
<td>IAM</td>
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<td>NS</td>
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<td>TPM</td>
<td>0.945</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>SMM</td>
<td>0.977</td>
<td>NS</td>
</tr>
<tr>
<td>INB</td>
<td>IAM</td>
<td>0.055</td>
<td>NS</td>
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<td>TPM</td>
<td>0.945</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>SMM</td>
<td>0.977</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, no significant
### Table 1. Primers used to develop the mitochondrial 16S rRNA gene in *A. purpuratus.*

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence (5'–3')</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S R</td>
<td>CCGRTYTGAACTCAGCTCACG</td>
<td>Puslednik and Serb (2008)</td>
</tr>
<tr>
<td>16S arl</td>
<td>CGCCTGTTTAACAAAAACAT</td>
<td>Palumbi et al. (1991)</td>
</tr>
<tr>
<td>ND1E-forward CO1AB-reverse</td>
<td>CGGCTTCGCCATGATCttyatygcnga</td>
<td>This study</td>
</tr>
<tr>
<td>ND1EAA</td>
<td>GATAAGGTTTTTGGGCATC</td>
<td>This study</td>
</tr>
<tr>
<td>16SAA</td>
<td>GGTCCACCTAGAAGCTAATG</td>
<td>This study</td>
</tr>
<tr>
<td>16SBB</td>
<td>GGTAAAGCGTGCTAAGGTA</td>
<td>This study</td>
</tr>
<tr>
<td>16SCC</td>
<td>GCGTAATCCGTCTTGACAGT</td>
<td>This study</td>
</tr>
</tbody>
</table>
Table 2. Microsatellites loci used to analyze the *A. purpuratus* population structure in this study.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Motif</th>
<th>Fluorescent label</th>
<th>$T_A$ (°C)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>APPE9</td>
<td>Trinucleotide</td>
<td>VIC</td>
<td>56</td>
<td>Marín et al. (2012)</td>
</tr>
<tr>
<td>APPE11</td>
<td>Trinucleotide</td>
<td>FAM</td>
<td>56</td>
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