Aquat. Living Resour. 23, 199–207 (2010)

DOI: 10.1051/alr/2010011
www.alr-journal.org

Aquatic Living Resources

Isolation and characterization of microsatellite markers in the queen scallop *Aequipecten opercularis* and their application to a population genetic study

Alberto Arias, Ruth Freire, Josefina Méndez and Ana Insua[a]

Departamento de Biología Celular y Molecular, Universidade da Coruña, A Zapateira s/n, 15071 A Coruña, Spain

Received 31 March 2009; Accepted 23 February 2010

Abstract – Microsatellites are one of the most popular markers in genetic studies but typically they need to be isolated and characterized *de novo* for each species. In this work, a genomic library enriched for a trinucleotide motif was constructed to identify polymorphic microsatellite loci in *Aequipecten opercularis*, a scallop species commercially fished in Europe, and to examine the level of genetic variation and genetic differentiation in samples from Spain and Northern Ireland. Sequencing of 83 clones led to the identification of 30 microsatellite-containing sequences which showed often other repeated sequences. Five microsatellite loci were successfully amplified and found polymorphic. The number of alleles and the expected heterozygosity per locus ranged from 9 to 86 and 0.341 to 0.927, respectively, all localities showing similar levels of genetic variation (allelic richness, 13.164–15.487; expected heterozygosity, 0.527–0.638). Discrepancies in genotype proportions from Hardy-Weinberg equilibrium were observed in 11 out of 25 locality-locus combinations, a heterozygote deficiency occurring in all cases probably due to null alleles. Significant genetic differentiation was detected among *A. opercularis* from Northern Ireland, Fuengirola (southern Spain) and the homogeneous samples from northwest Spain. Isolation by distance was the most likely hypothesis to explain the differentiation detected.

Key words: *Aequipecten opercularis* / Genetic variation / population differentiation / Microsatellite marker / Queen scallop

Résumé – Les microsatellites sont parmi les marqueurs les plus utilisés dans les études génétiques mais ils nécessitent d'être isolés et caractérisés *de novo* pour chaque espèce. Dans cette étude, une bibliothèque génomique, enrichie pour un motif trinucléotidique, est construite pour identifier ces microsatellites polymorphes chez *Aequipecten opercularis*, une espèce de Pectinidés (vanneau) pêchée en Europe, et pour examiner le niveau de variation génétique et de différentiation génétique chez des échantillons provenant d'Espagne et d'Irlande du Nord. Le séquençage de 83 clones conduit à l'identification de 30 séquences présentant des microsatellites qui, souvent, montrent d'autres séquences répétées. Cinq loci microsatellites sont amplifiés avec succès et s'avèrent polymorphes (taux de variabilité important d'un individu à l'autre). Le nombre d'allèles et l'hétérozygosité attendue par locus s'étend de 9 à 86 et de 0,341 à 0,927, respectivement ; tous les sites géographiques montrant des niveaux similaires de variation génétique (richesse allélique : 13,164–15,487 ; hétérozygosité attendue : 0,527–0,638). Des différences dans les proportions de génotype sont observées par rapport à l'équilibre de Hardy-Weinberg dans 11 des 25 combinaisons « sites géographiques-locus », une déficience hétérozygote est détectée dans tous les cas, probablement due aux allèles nuls. Une différentiation génétique significative est détectée parmi les échantillons de *A. opercularis* provenant d'Irlande du Nord, de Fuengirola (sud de l'Espagne), et des échantillons homogènes du nord-ouest de l'Espagne. L'isolation due à la distance est l'hypothèse la plus probable pour expliquer ces différences détectées.

Introduction

The queen scallop *Aequipecten opercularis* (Linnaeus 1758) is a simultaneous hermaphrodite species of the bivalve family Pectinidae with external fertilization, a larval planktonic stage of several weeks and adults that can swim (Cragg and Crisp 1991). It is distributed along the European coast, from Norway to northwest Africa, including the Mediterranean, where it may be found in great abundance on muddy or sandy bottoms from infralittoral down to 400 m (Wagner 1991). The maximum growth in shell width is about 90 mm and it is commercially fished in Ireland, UK, France and Spain (Brand 2006), representing one of the European scallop species with the highest catch volume.

[a] Corresponding author: insuax@udc.es

The assessment of genetic variation and population differentiation is important for identifying management units and maintaining sustainable fisheries. The existence of significant geographic heterogeneity indicates that populations could be predominantly self-recruiting and overexploitation could eliminate the broodstock. Moreover, self-recruiting populations may become locally adapted and the widespread practice of stock enhancement by extensive transplantation from other areas could reduce the mean population fitness. Population genetic studies in *A. opercularis* have been carried out using mostly electrophoresis of allozymes which detect genetic variation at protein level (Beaumont 2006). The available estimates of genetic variability in *A. opercularis*, determined as proportion of polymorphic loci and mean observed heterozygosity, take an intermediate value among scallop species (Beaumont 2006). Population differentiation has been investigated in several instances. Mathers (1975) reported one locus with significant differences in the distribution of phenotypes and allele frequencies between two samples from the east and west coasts of Ireland, and Beaumont (1982), after the analysis of four gene loci, proposed that around the British Isles there are at least four relatively genetically isolated populations. While no significant genetic differentiation was found by Beaumont (1982) and Macleod et al. (1985) in the samples from the Irish Sea, Lewis and Thorpe (1994) reported that populations sampled from around the UK, most of them in the Irish Sea, are genetically heterogeneous. DNA-based markers such as mitochondrial genes, intron polymorphisms, and single nucleotide polymorphisms (SNPs) were used to analyze samples from Spain and Northern Ireland, reporting estimates of genetic variability and lack of genetic differentiation among Spanish samples (Fernández-Moreno et al. 2008; Arias et al. 2009a; Arias et al. 2009b). Although biological reasons may explain the absence of genetic differentiation, it cannot be discarded that this may be related to the power of the markers used to detect variation.

Microsatellites, or simple sequence repeats (SSRs), are tandemly repeated motifs of 1-6 bases that span less than a few hundred bases (Chambers and MacAvoy 2000). They are found in all genomes mostly in noncoding regions and are usually characterized by a high degree of polymorphism due to variation in the number of tandem repeats (Li et al. 2002). Although some microsatellites have functional relevance, typically they represent selectively neutral markers and show a co-dominant mode of inheritance, they can be easily amplified by polymerase chain reaction (PCR) and they are highly reproducible. They have proven to be valuable for research in different areas, including genetic mapping, individual identification, parentage assignment, genetic dissection of complex traits and population and conservation genetics (Chistiakov et al. 2006). In the field of population genetics and management of marine resources their use has revealed the existence of genetic structuring in species previously thought to be homogeneous over large geographical ranges (Jørgensen et al. 2005; Was et al. 2008) and differentiation at fine-scale in others (Cabranes et al. 2008; Diz and Presa 2008). The principal drawback of microsatellites is that usually they must be isolated and characterized in each species given that the flanking sequences are not often conserved across species.

In this work we report the isolation of microsatellite sequences in *A. opercularis* and the characterization of five polymorphic loci. These new markers were used to examine five localities of Spain and Northern Ireland, providing new estimates of genetic variation and population differentiation.

Material and methods

Specimen collection and DNA extraction

Specimens of *A. opercularis* were collected from five sites: O Grove (42°29′58"N, 8°51′55"W), Cambados (42°30′44"N, 8°49′24"W) and San Simon-Rande (SS-Rande; 42°17′43"N, 8°37′22"W) in northwest Spain (Atlantic coast), Fuengirola (36°30′39"N, 4°35′35"W) in southern Spain (Mediterranean coast) and Antrim (55°14′53"N, 6°21′1"W) in Northern Ireland. Total genomic DNA was extracted from a 30-mg piece of ethanol-preserved adductor muscle according to Fernández-Tajes and Méndez (2007) after two 15-min washes in phosphate buffered saline and sterile deionized water.

Enriched library construction

A TTC sequence enriched library was constructed according to Billote et al. (1999). In brief, total genomic DNA was digested with *Rsa*I restriction enzyme and the fragments obtained were ligated to *Rsa* linkers. To obtain more product and check the ligation, a PCR was carried out using one of the *Rsa* linkers as primer (Edwards et al. 1996). Next, the fragments were selected using streptavidin-coated magnetic particles (Magnesphere, Promega) and a 5'-biotinylated $(TTC)_6$ oligonucleotide probe. The enriched single-stranded DNA was amplified to obtain more product and double-stranded DNA. The product of the PCR amplification was cloned into pCR®2.1-TOPO® plasmids using the kit TOPO TA Cloning® (Invitrogen). Recombinant clones were transferred to Hybond™-N+ nylon membranes (Amersham Biosciences) and screened by hybridization using a $(GAA)_7$ digoxigenin-labeled probe. The plasmid DNA of positive clones was extracted using the QUIAprep® Spin Miniprep Kit (Quiagen) and the sequence of the inserts determined at the Molecular Biology Unit of the University of A Coruña (Spain) using a CEQ™ 8000 (Beckman Coulter) or ABI PRISM 3130xl (Applied Biosystems) automated sequencer. The nucleotide sequences of clones containing microsatellites have been deposited in the EMBL database under accession numbers FM202330-FM202360.

Sequence analysis

Sequences were analyzed for the presence of microsatellites with the help of the program Tandem Repeats Finder (Benson 1999). The similarity between the sequenced clones was checked by means of an all-against-all comparison using the local BLAST tool of the program BioEdit v. 7.0.9.0 (Hall 1999), selecting the option of filter sequences for low-complexity regions. Sequences containing microsatellites

were also compared with those of public databases using the discontiguous megablast algorithm of the BLAST tool (Altschul et al. 1997). Similarity among microsatellite flanking regions was checked with the program MicroFamily (Meglécz 2007).

Detection of microsatellite polymorphism

Oligonucleotide primers flanking the microsatellite regions were designed using the program OLIGO v. 3.4 (Rychlik and Rhoads 1989). Initial optimization reactions, when necessary, were carried out in a gradient thermalcycler. Approximately 40 ng of template DNA were used in a reaction volume of 12.5 μl containing 0.3 U of *Taq* DNA polymerase in 1X reaction buffer (Roche Molecular Biochemicals), 0.25 mM of each dNTP, 0.3 μM of each primer and $MgCl_2$ ranging from 1.5 to 3.0 mM. The thermal cycler protocol consisted of an initial denaturation of 2 min at 95 °C, followed by 35 cycles of 95 °C for 45 s, 48–65 °C for 45 s, and 72 °C for 2 min, and a final extension of 72 °C for 20 min. The PCR products were checked in 2% agarose gels and/or 6% polyacrylamide, followed by ethidium bromide or silver staining, respectively. Routine amplifications were performed in a 25 μl volume, with the reagent concentrations and thermalcycler protocol previously described but with 80 ng of template DNA, 0.6 U of *Taq* DNA and the $MgCl_2$ concentration and annealing temperature determined for each microsatellite locus (see Results section). The forward primer was 5' labeled with one fluorescent phosphoramidite dye, 6-FAM or HEX, and the amplified products were run on an ABI PRISM 3130xl and analyzed using the GeneMapper v. 3.7 software (Applied Biosystems).

Population genetic analysis

The number of alleles, the observed heterozygosity (H_o) and the unbiased expected heterozygosity (H_e) of Nei (1978) were obtained with the Genetix software v.4.03 (Belkhir et al. 2004). Allelic richness (Rs) per locus, population and overall was computed with Fstat v. 2.9.3 (Goudet 2001). Tests for agreement with Hardy-Weinberg equilibrium, allelic differentiation and linkage disequilibrium between pairs of loci within each population were carried out with Genepop v.3.4 software (Raymond and Rousset 1995). The significance was determined by a Markov chain method using 10 000 dememorizations, 1000 batches and 5000 iterations per batch, except when the Hardy-Weinberg equilibrium was tested for samples with fewer than five alleles where the complete enumeration method (Louis and Dempster 1987) was applied. A Friedman test was carried out to compare the allelic richness among localities with the statistical package SPSS 14.0 (SPSS Inc.). The program MicroChecker (Van Oosterhout et al. 2004) was used to examine the microsatellite data for evidence of null alleles whose frequency was estimated following Brookfield (1996).

Single locus and multilocus F-statistics were computed with Genetix for each sample and for all individual loci, as well as for all pairs of sampling sites, according to Weir and Cockerham (1984). Probability of significance of θ values was determined by a non-parametric permutation approach (10 000 permutations). When multiple tests were performed the significance values were adjusted using the sequential Bonferroni correction (Rice 1989).

To test for isolation by distance, a Mantel test was performed in IBDWS v. 3.15 (Jensen et al. 2005) using 10 000 randomizations. The genetic differentiation between localities was measured as $\theta/(1\text{-}\theta)$ and the geographical distance (km) as the coastline distance between sample locations and also as the lowest marine distance. A hierarchical analysis of molecular variance (AMOVA), locus by locus, was conducted using the software Arlequin 3.11 (Excoffier et al. 2005) to assess the component of genetic diversity attributable to variance among groups (different regional groupings were tested), variance among localities within groups and variance within localities.

Results

Isolation and characterization of microsatellite markers

The construction of a TTC enriched library yielded 1100 recombinant colonies of which 83 showed hybridization with the $(GAA)_7$ probe. After sequencing, 70 clones, with an insert size of 202 to 1281 bp, contained at least one microsatellite. Thirty of the microsatellite-containing sequences were unique and displayed the expected TTC repeats and/or other repeated motifs (TTA, TTG, TAT, TTTA or TATG) (Table 1). Following Chambers and MacAvoy (2000), the microsatellites were classified as pure (15), compound (1), interrupted pure (14) and interrupted compound (6). Higher-order tandem repeats were also found in five clones containing microsatellites (Table 1).

The local BLAST tool of the program BioEdit revealed that most of the clones showed a certain degree of similarity with others, and using the program MicroFamily 13 clones were assigned to three groups of sequences related by one of the flanking regions. When the sequences were compared to those of public databases, one flanking region of *Aop*69 showed similarity with a microsatellite-containing sequence of *Chlamys farreri* (E-value = 2×10^{-17}; accession: EF017119) and the two flanking regions of *Aop*22 showed similarity with those of a *Solen marginatus* microsatellite (E-value $\leqslant$ 6×10^{-10} accession: AM422783).

Twenty-one of the 30 unique sequences showing microsatellite repeats were selected for primer design, based on the length of the microsatellite and on the availability of flanking regions. Five microsatellite loci were successfully amplified and found polymorphic. The optimal annealing temperatures and $MgCl_2$ concentrations are indicated (Table 2). The other primer sets (16) yielded non-scorable products due to excessive "stutter", apparent amplification of multiple loci (more than two bands), and/or failure to amplify DNA from a large number of individuals, despite attempts of optimization (including design of new primers).

Population genetic variation

Genetic variation statistics by locus, population and overall are shown (Table 3). The number of alleles in the five

Table 1. Microsatellite sequences of *Aequipecten opercularis* (Mollusc: Pectinidae) identified on the unique clones.

Clone	Repeat	Class	Accession
*Aop*02*	$(TTA)_3N_{42}(TTC)(TTA)_2TT(TTC)_{23}TT(TTC)_{14}$	P; IP	FM202330
*Aop*03	$(AT)_4N_{615}(AT)_4N_{82}(TTC)TATC(TTC)_{35}$	P; P; IP	FM202331
*Aop*04	$(TTG)_3$	P	FM202332
*Aop*07	$(TTA)_5AGT(C)_4(TTA)N_{18}(TTA)_4(TTC)_2CGGC(TTC)_{12}$	IP; IC	FM202333
*Aop*08	$(TTC)_3CTC(TTC)TCT(TTC)TTT(TTC)TTT(TTC)_{35}N_{45}(TATG)_3$	IP; P	FM202334
*Aop*09	$(TTC)_{16}$	P	FM202335
*Aop*10	$(TTC)_{32}CTC(TTC)_{29}$	IP	FM202336
*Aop*11	$(TTC)_{23}TCC(TTC)_{18}$	IP	FM202337
*Aop*12	$(TG)_4A(TG)_2T(TG)N_{537}(TTA)_5(TTC)CGG(TTC)_{47}$	IP; IC	FM202338
*Aop*14	$(TTC)_6$	P	FM202339
*Aop*22	$(TTC)_{135}$	P	FM202340
*Aop*24	$(TTA)_3(TTC)_{10}$	C	FM202341
*Aop*25	$(TTC)^a_{209}$	IP	FM202342
*Aop*27	$(TTG)_3$	P	FM202343
*Aop*29	$(TTC)(TTA)_2(TTC)_{11}$	IP	FM202344
*Aop*31*	$(TTC)_{14}TC(TTC)_4$	IP	FM202345
*Aop*32	$(TAT)_3AATGATTA(TAT)CGA(TAT)TAG(TAT)$	IP	FM202346
*Aop*35*	$(TTC)_{37}$	P	FM202347
*Aop*37	$(TTC)_{34}$	P	FM202348
*Aop*40	$(TTC)_{35}TCC(TTC)_{14}TTT(TTC)_2TTA(TTC)_3TTA(TTC)_2(T)_5(TTC)$	IP	FM202349
*Aop*41	$(TTC)_{26}$	P	FM202350
*Aop*42*	$(TTA)_3(TTC)_2AGGC(TTC)_{11}CTC(TTC)_2$	IC	FM202351
*Aop*45	$(TTA)_4(TTC)(T)_4(TTC)GTC(TTC)_9$	IC	FM202352
*Aop*52	$(TTA)_5(TTC)TTT(TTC)TGCCTA(TTC)_5CTC(TTC)_{43}TCC(TCC)_{16}$	IC	FM202354
*Aop*53	$(TTA)_3(TTC)_2TAC(TTC)_2TT(TTC)_{32}$	IC	FM202355
*Aop*67	$(ATT)_3ACTA(ATT)$	IP	FM202356
*Aop*68*	$(TTC)_{22}$	P	FM202357
*Aop*69	$(TTTA)_3$	P	FM202358
*Aop*74	$(TTC)TTA(TTC)_8$	IP	FM202359
*Aop*78	$(TTC)_5$	P	FM202360

Pure (P); Interrupted pure (IP); Compound (C); Interrupted compound (IC)
*Clones containing higher-order tandem repeats
[a]: full microsatellite sequence of *Aop*25
$(TTC)_6TTG(TTC)_4TTG(TTC)_5TAC(TTC)TTG(TTC)_5TTG(TTC)_5TCC(TTC)_2TTG(TTC)_3TTG(TTC)_7TAC(TTC)TTG(TTC)_7$
$TTG(TTC)_5TTG(TTC)_3TTG(TTC)TTG(TTC)_5TAC(TTC)TTG(TTC)_4TTG(TTC)_9TTG(TTC)_5TTG(TTC)_3TTG(TTC)TCC(TTC)_7$
$TTG(TTC)_3TAC(TTC)_{16}TAC(TTC)TTG(TTC)_7TTG(TTC)_2TCC(TTC)TTG(TTC)_2TTG(TTC)_2TTG(TTC)_4TTG(TTC)_2$
$TAC(TTC)TTG(TTC)_{11}TTG(TTC)_{34}$

Table 2. PCR annealing temperatures and $MgCl_2$ concentrations for optimum amplification of five microsatellite loci in *A. opercularis*.

Locus	Primers (5' to 3')	T_a (°C)	$MgCl_2$ (mM)
*Aop*02	GCGTTTCTTATTATTCTTCTTC TCGAGAAATCGTGCGGACAA	56	2.0
*Aop*14	TCGTCTCCGTTATTAGGC GAGATCAACTCCATTACG	60	2.0
*Aop*29	GCTTTTTACGTGTCTTTCAG AAATCACGGCGGAAGAAGAA	58	2.0
*Aop*45	AAGTTCCCCAAACGAAGTGA AAGTTTCTTGCACCCCTCCT	60	1.5
*Aop*52	GGTGGGGACTCTTTAGCGT TCTCCTTACCATCCCAAATAC	60	1.5

polymorphic loci ranged from nine (*Aop*02) to 86 (*Aop*52). All loci displayed alleles differing by multiples of three base pairs except *Aop*14, where some alleles showed size increments of 1 or 2 bp. Forty two of the 146 alleles observed were private to the sampled populations with a mean frequency of 1.3% (range: 0.7–8.7%), the locus *Aop*52 displaying the highest number with 23 and *Aop*14 and *Aop*29 the lowest with four. Although the number of private alleles tended to increment with sample size, the correlation was non-significant ($F_{[1,3]} = 6.151$, $p = 0.089$, $R^2_{\text{adjusted}} = 0.563$). Allelic richness ranged across localities from 3.264 (*Aop*02) to 39.287 (*Aop*52) and for each locus-locality pair between 2.880 and 40.355. The mean number of alleles per locality was 13.400 (O Grove) to 21.400 (Cambados) but the mean allelic richness was 13.164 (Antrim) to 15.487 (Cambados). A Friedman test (p-value = 0.525) was unable to detect significant differences in the allelic richness among localities. Expected heterozygosity per locus was 0.141 (*Aop*02) to 0.984 (*Aop*52) and the observed heterozygosity was between 0.148 (*Aop*02) and 0.854 (*Aop*29). All localities showed similar values of H_e and H_o, ranging from 0.527 (Antrim) to 0.638 (Fuengirola) and from 0.418 (O Grove) to 0.474 (Fuengirola), respectively. Of the

50 tests for linkage disequilibrium carried out, none were significant at $p < 0.05$, indicating that the loci are not closely linked and that they can be treated as independent variables.

Fifteen out of the 25 locality-locus combinations deviated from Hardy-Weinberg equilibrium ($p < 0.05$), with 11 of these being significant after sequential Bonferroni correction (Table 3). Four loci were in Hardy-Weinberg equilibrium in all or at least one locality, the locus *Aop*52 departing from it in all localities. Deviations were always caused by a heterozygote deficit ($f \geqslant 0.230$). The estimation of f within populations across loci was always positive, ranging from 0.188 in Antrim to 0.340 in O Grove. The locality-locus f values were also positive, except for *Aop*02 in all localities and *Aop*45 in Antrim (Table 3). The overall value by locus ranged from –0.054 for *Aop*02 to 0.487 for *Aop*14. According to the program MicroChecker null alleles were absent in the locus *Aop*02, but in the other loci they occurred in at least two localities. *Aop*14 and *Aop*52 were the most affected loci with null alleles in four and five sites, respectively. The estimated null allele frequencies were always less than 0.200 except for *Aop*14 in Cambados (0.275) and O Grove (0.286). Taking into account that the presence of null alleles at high frequencies (> 0.2) can lead to a considerable overestimation of F_{ST} estimators and genetic distance (Chapuis and Estoup 2007) the locus *Aop*14 was removed from the subsequent analyses.

Population genetic differentiation

The global multilocus θ value was 0.006, significantly different from zero ($p = 0.001$). Estimations of θ per locus ranged from –0.003 (*Aop*02) to 0.034 (*Aop*45) with $p = 0.001$ for the *Aop*45 locus. Five of the 10 pairwise θ values were significant at $p < 0.05$, and two after sequential Bonferroni correction (Antrim-O Grove and Antrim-Fuengirola), Antrim and Fuengirola showing the maximum differentiation ($\theta = 0.026$) (Table 4). Of the 40 pairwise tests for population differentiation using allelic frequencies, 16 were significant at $p < 0.05$ and three (locus *Aop*45: Antrim-all localities, except Cambados) after sequential Bonferroni correction (Table 4). The Mantel test revealed a significant correlation between genetic and geographical distances ($R^2 \geqslant 0.811$; $p \leqslant 0.035$), suggesting a pattern of isolation by distance. A hierarchical, locus by locus, AMOVA grouping the localities based on a geographical criterion (Table 5) showed that a significant part of the total variance corresponded to the among groups component (1.1%). Most of the variance was distributed between individuals within localities (98.88%) and a very small percentage (0.04%) corresponded to localities within groups. When samples from northwest Spain were grouped with Antrim or Fuengirola, the component of among localities within groups was significant.

Discussion

Microsatellite sequences can be isolated by different methods (reviewed by Zane et al. 2002). The most straightforward consists of the screening of databases, however, it is not applicable to organisms with few available nucleotide sequences. In this work an enriched library was constructed after selection of DNA fragments with a TTC probe. This method is relatively simple, efficient and widely applicable, leading for the first time in *A. opercularis* to the isolation of 30 microsatellite-containing sequences and the characterization of five polymorphic microsatellite loci.

The number of polymorphic microsatellite loci obtained with respect to the total sequences examined in *A. opercularis* (6.0%) is higher than that yielded by enriched libraries constructed in the scallop *Mizuhopecten yessoensis* (4.6%, An et al. 2005), the oyster *Crassostrea virginica* (2.4%, Reece et al. 2004) or the pearl oyster *Pinctada maxima* (0.2%, Evans et al. 2006), and lower than that obtained in other scallops, *Chlamys farreri* (12.5%, Zhan et al. 2007), *Pecten maximus* (15.8%, Watts et al. 2005) and *C. nobilis* (27.5%, Ma and Yu 2009) or the oyster *C. gigas* (19.6%, Li et al. 2003). The efficiency of the isolation of microsatellites as usable markers might be affected by both biological and technical factors. It is known that the frequency of microsatellites differs among taxa. In bivalves, closely related species show remarkable differences in microsatellite density and some microsatellite motifs show one of the lowest genomic densities observed among eukaryotes (Cruz et al. 2005). Due to the enriched nature of the constructed library, it is not possible to estimate the frequency of the TTC microsatellite in *A. opercularis* but it is likely that the relatively low number of clones containing microsatellites results from the low representation in the genome. When enrichment procedures are used, as was the case here, repeated clones are often isolated (Zane et al. 2002). Here the redundancy obtained was relatively high (48%), reducing the number of available sequences for primer design. Also, the structure of the flanking regions influences the obtaining of usable markers (Nève and Meglécz 2000). In this work, significant similarity was found among most of the sequences obtained and 13 of the unique sequences contained related stretches on one flanking region, indicating that the TTC microsatellite motif of *A. opercularis* is frequently linked to other sequences repeated in the genome. This also reduced the stretch available for primer design and may originate multiple locus amplifications and unclear banding patterns. Moreover, the intraspecific polymorphism of flanking regions could affect the rate of success of primers given that some primer sets of *A. opercularis* do not yield DNA amplifications in a large number of individuals.

The microsatellite loci characterized in *A. opercularis* showed a higher level of genetic variation than allozyme loci or other DNA nuclear markers examined in previous studies. In all microsatellite loci the number of alleles ranged from nine for *Aop*02 to 86 for the hypervariable locus *Aop*52, in contrast to the 2-6 alleles observed with allozymes (Beaumont and Beveridge 1984) or 3 alleles with other DNA nuclear markers (Arias et al. 2009a). This is an expected result taking into account that microsatellites are characterized by a high mutation rate. Although the localities examined differ in their exploitation regimen, *A. opercularis* is regularly fished in Cambados and O Grove for example, while in Fuengirola there is not commercial exploitation, all localities showed similar levels of genetic variation. Compared to other bivalves, the mean number of alleles per locus and per locality (17.1) was in the range

Table 3. Summary statistics of genetic variation by locus, population and overall and *P*-value of the test for conformity to Hardy-Weinberg expectations at five microsatellite loci for five *A. opercularis* localities.

		Locality					Overall
Locus		Antrim	Cambados	OGrove	SS-Rande	Fuengirola	
*Aop*02	*N*	39	75	33	68	48	263
	N_A	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)	9 (5)
	R	157-184	178-187	157-184	139-184	151-184	139-187
	R_S	3.528	2.967	3.818	2.88	3.569	3.264
	A_C	181	181	181	181	181	181
	Freq AC	0.936	0.947	0.924	0.912	0.906	0.926
	H_e	0.124	0.103	0.146	0.164	0.177	0.141
	H_o	0.128	0.107	0.152	0.177	0.188	0.148
	f	–0.035	–0.034	–0.042	–0.074	–0.061	–0.054
	p-value	1.000	1.000	1.000	1.000	1.000	
*Aop*14	*N*	48	69	32	57	38	244
	N_A	9 (1)	13 (2)	8	11 (1)	8	15 (4)
	R	123-139	123-142	123-136	126-141	123-136	123-142
	R_S	8.174	10.987	7.872	9.130	7.284	9.831
	A_C	129	129	129	129	129	129
	Freq AC	0.667	0.435	0.453	0.684	0.605	0.570
	H_e	0.540	0.764	0.718	0.520	0.579	0.646
	H_o	0.375	0.275	0.219	0.316	0.447	0.324
	f	0.308	0.641	0.699	0.395	0.230	0.487
	p-value	<0.001 ∗ ∗	<0.001 ∗ ∗	<0.001 ∗ ∗	<0.001 ∗ ∗	0.189	
*Aop*29	*N*	52	75	31	68	48	274
	N_A	16	19 (3)	14	17	18 (1)	22 (4)
	R	132-180	129-198	135-177	132-180	135-186	129-198
	R_S	14.930	16.176	13.935	15.495	16.194	15.954
	A_C	153, 156, 159	156	135	153	144	156
	Freq AC	0.125	0.140	0.145	0.126	0.188	0.117
	H_e	0.924	0.926	0.928	0.928	0.915	0.927
	H_o	0.923	0.813	0.871	0.838	0.854	0.854
	f	0.001	0.122	0.063	0.097	0.067	0.077
	p-value	0.080	0.105	0.296	0.030*	0.008*	
*Aop*45	*N*	43	62	30	57	44	236
	N_A	3	10 (5)	5	7 (1)	8	14 (6)
	R	184-193	127-211	187-199	186-202	184-202	127-211
	R_S	2.609	6.952	5.000	4.883	7.416	5.925
	A_C	187	187	187	187	187	187
	Freq AC	0.965	0.798	0.783	0.816	0.659	0.805
	H_e	0.069	0.355	0.375	0.319	0.537	0.341
	H_o	0.070	0.290	0.233	0.175	0.227	0.203
	f	–0.016	0.184	0.382	0.453	0.580	0.388
	p-value	1.000	0.031*	0.037*	<0.001 ∗ ∗	<0.001 ∗ ∗	
*Aop*52	*N*	57	72	31	65	46	271
	N_A	50 (2)	61 (8)	36 (2)	55 (8)	40 (3)	86 (23)
	R	186-465	168-462	171-405	168-450	207-465	168-465
	R_S	36.580	40.355	35.350	38.246	34.100	39.287
	A_C	255	237	264, 273, 309	273	243, 342	237
	Freq AC	0.070	0.056	0.065	0.054	0.054	0.037
	H_e	0.980	0.985	0.979	0.983	0.980	0.984
	H_o	0.649	0.667	0.613	0.723	0.652	0.668
	f	0.339	0.325	0.378	0.266	0.337	0.322
	p-value	<0.001 ∗ ∗	0.001**	<0.001 ∗ ∗	<0.001 ∗ ∗	<0.001 ∗ ∗	
All loci	*N*	59	75	33	68	53	288
	Mean N_A	16.400	21.400	13.400	18.800	15.600	
	Mean Rs	13.164	15.487	13.195	14.127	13.713	
	H_e	0.527	0.627	0.629	0.583	0.638	0.608
	H_o	0.429	0.431	0.418	0.446	0.474	0.440
	f	0.188	0.315	0.340	0.237	0.259	0.271

N, number of individuals; N_A, observed number of alleles (private alleles); *R*, size range of alleles in base pairs; R_S, allelic richness based on a minimum sample size of 30 diploid individuals; A_C and Freq A_C, size in base pairs and frequency of the most common allele; H_e, the expected heterozygosity based on Nei's unbiased estimate; H_o, the observed heterozygosity; *f*, the inbreeding coefficient estimated following Weir and Cockerham (1984); *Significant at 5% level; ** Significant after sequential Bonferroni correction.

Table 4. Test for genetic differentiation between five localities of *A. opercularis* based on the θ estimator (below diagonal) and allele frequencies (above diagonal, only loci displaying significant differences are shown).

Locality	Antrim	Cambados	OGrove	SS-Rande	Fuengirola
Antrim		*Aop*45*	*Aop*29*, *Aop*45**	*Aop*45**, *Aop*52*	*Aop*45**
Cambados	0.006*			*Aop*52*	*Aop*29*, *Aop*45*, *Aop*52*
OGrove	0.012**	–0.003		*Aop*45*, *Aop*52*	*Aop*29*, *Aop*52*
SS-Rande	0.007*	–0.001	–0.001		*Aop*29*, *Aop*52*
Fuengirola	0.026**	0.006*	0.006	0.006	

*p-values significant at $p < 0.05$.
** p-values significant after sequential Bonferroni correction.

Table 5. Analysis of molecular variance describing the partitioning of genetic variation for the five localities of *A. opercularis.* Three groups (Antrim, Fuengirola and localities of northwest Spain) were established according to a geographical criterion.

Source of Variation	Sum of Squares	Variance components	Percentage variation	p-value
Among groups	5.896	0.013	1.09	0.014
Among localities within groups	2.471	0.000	0.04	0.379
Within localities	628.527	1.189	98.88	<0.001
Total	636.894	1.202		

Fixation indices: $F_{CT} = 0.012$, $F_{SC} = 0.012$, $F_{ST} = 0.014$.

observed for other species such as the oyster *Ostrea edulis* (18.5, Launey et al. 2002), the clam *Panopea abrupta* (29.9, Vadopalas et al. 2004) or the mussel *Mytilus galloprovincialis* (11.9, Diz and Presa 2008) but the overall expected heterozygosity (0.608) was lower (0.930, 0.94 and 0.770, respectively), although high expected heterozygosity values ($H_e > 0.90$) were observed for the loci *Aop*29 and *Aop*52.

In 11 out of 25 locus-locality combinations, a large heterozygote deficit relative to Hardy-Weinberg equilibrium was observed. This is a common observation in marine bivalve studies, first observed with allozymes (e.g. Gaffney 1994) but also reported with DNA markers such as microsatellites (e.g. Launey et al. 2002). Both biological (inbreeding, Wahlund effect or selection) and technical explanations (null alleles) have been proposed. When inbreeding exists, a similar increase in homozygosity at every locus is expected, which was not the case. The Wahlund effect, a deficiency of heterozygotes due to the existence of substructure within samples, cannot be a major contributor to the homozygote excess since large differences in allele frequencies among subsamples should occur, this being reflected in higher θ values than those observed. The action of natural selection cannot be discarded. Although most microsatellite loci are assumed to be neutral, some may be under the direct or indirect effect of selection (Li et al. 2002; Boudry et al. 2002). However, null alleles, which are non-amplifying alleles usually caused by mutations in the primer annealing sites, are the most probable cause of the heterozygote deficiencies. When null alleles are present, heterozygotes bearing null alleles will be mistyped as homozygotes and this scoring error can cause a false observed heterozygote deficiency in the population. The frequency of null alleles that would explain the deficiencies observed here ranged from 0.043 to 0.286. Similarly, null alleles have been detected at microsatellite loci in other bivalves (Reece et al. 2004; Li et al. 2003; Launey et al. 2002; Vadopalas et al. 2004; Kenchington et al. 2006; Zhan et al. 2009). Given that mutations affecting priming sites do not necessary affect all populations equally, it is possible to find samples with and without an apparent deficit of heterozygotes as occurs with the loci *Aop*14 and *Aop*45.

The global θ value obtained in this study ($\theta = 0.006$, p-value = 0.001) although low was highly significant, indicating the existence of genetic differentiation. For microsatellite loci, obtaining low estimates of θ is expected because the high polymorphism can greatly reduce the estimates of F_{ST} values even in the absence of shared alleles (Balloux and Lugon-Moulin 2002). Both the pairwise θ value and allelic differentiation tests for locus *Aop*45 revealed significant differences between Antrim and Fuengirola, the two localities that are geographically more distant, as well as between Antrim and O Grove. Significant differences were also detected between Antrim and San Simon Rande in the allelic frequencies of locus *Aop*45. The AMOVA indicated homogeneity among the localities of northwest Spain and significant differences among these, Antrim and Fuengirola. This is congruent with the slightly negative pairwise θ values observed in comparisons involving Cambados, O Grove and San Simon-Rande and a θ value of at least 0.006 when these localities are compared with Antrim or Fuengirola. Globally, the data obtained suggest that localities of Northern Ireland, northwest and southern Spain are weakly structured. Isolation by distance is the most likely hypothesis to explain the differentiation among localities given a significant correlation was observed between genetic and geographical distances. This picture contrasts with the genetic homogeneity revealed by the analysis of mitochondrial genes (Fernández-Moreno et al. 2008) and intron polymorphisms (Arias et al. 2009a) in the same localities but corroborates the existence of differentiation between Antrim and Spanish samples revealed previously by SNP markers (Arias et al. 2009b) and demonstrates that microsatellite markers are adequate to detect subtle genetic differentiation. In the context

of the Spanish coast, the results obtained here for *A. opercularis* are in line with those described for the scallops *Mimachlamys varia* (Fernández-Moreno et al. 2008), *Pecten maximus* (Ríos et al. 2002) or the mussel *M. galloprovincialis* (Diz and Presa 2008; Quesada et al. 1995) regarding the genetic homogeneity of northwest localities and the existence of small differentiation with respect to Fuengirola or other nearby localities. Nevertheless, at least in the case of *M. galloprovincialis*, the populations better fit an island model nuanced by random migration than an isolation by distance model (Diz and Presa 2008). A different larval dispersal capacity, less extensive both spatially and temporally in *A. opercularis* than in *M. galloprovincialis*, may explain this discrepancy.

In conclusion, this work provides for the first time in *A. opercularis* the isolation of microsatellite sequences and the characterization of five polymorphic microsatellite markers useful for genetic studies. Moreover, it describes the level of genetic variation occurring in five localities of Spain and Northern Ireland and reveals the existence of genetic differentiation among samples previously considered homogeneous after the analysis of other molecular markers. The analysis of the microsatellite loci characterized here in samples from other European localities will provide a more accurate picture of the genetic structure of this species and consequently valuable information for management of the fisheries.

Acknowledgements. We thank Dr. Guillermo Román, Dr. Antonio Hervas and the Cangas and Cambados Association of Fishermen for supplying the scallop samples and Jose García Gil for the technical assistance. This work was supported by Ministerio de Ciencia y Tecnología through project AGL2003-07430.

References

Altschul S.F., Madden T.L., Schaffer A.A., Zhang J.H., Zhang Z., Miller W., Lipman D.J., 1997, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

An H.S., Park J.Y., Lee Y.G., Lee D.S., Lee C., 2005, Ten polymorphic microsatellite loci in the giant scallop (*Mizuhopecten yessoensis*). Mol. Ecol. Notes 5, 806–808.

Arias A., Freire R., Méndez J., Insua A., 2009a, Intron characterization and their potential as molecular markers for population studies in the scallops *Aequipecten opercularis* and *Mimachlamys varia*. Hereditas 146, 46–57.

Arias A., Freire R., Boudry P., Heurtebise S., Méndez J., Insua A., 2009b, Single nucleotide polymorphism for population studies in the scallops *Aequipecten opercularis* and *Mimachlamys varia*. Conserv. Genet. 10, 1491–1495.

Balloux F., Lugon-Moulin N., 2002, The estimation of population differentiation with microsatellite markers. Mol. Ecol. 11, 155–165.

Beaumont A., 2006, Genetics. In: Shumway S. E., Parsons G. J. (Eds.) Scallops: biology, ecology and aquaculture. Amsterdam, Elsevier, pp. 543–594.

Beaumont A.R., 1982, Geographic-variation in allele frequencies at three loci in *Chlamys opercularis* from Norway to the Brittany Coast. J. Mar. Biol. Assoc. UK 62, 243–261.

Beaumont A.R., Beveridge C.M., 1984, Electrophoretic survey of genetic variation in *Pecten maximus*, *Chlamys opercularis*, *Chlamys varia* and *Chlamys distorta* from the Irish Sea. Mar. Biol. 81, 299–306.

Belkhir K., Borsa P., Chikhi L., Raufaste N., Bonhomme F., 2004, GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier (France). Available from: http://www.genetix.univ-montp2.fr/genetix/genetix.htm.

Benson G., 1999, Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 27, 573–580.

Billote N., Lagoda P.J.L., Risterucci A.M., Baurens F.C., 1999, Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. Fruits 54, 277–288.

Boudry P., Collet B., Cornette F., Hervouet V., Bonhomme F., 2002, High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. Aquaculture 204, 283–296.

Brand A.R., 2006, The European scallop fisheries for *Pecten maximus*, *Aequipecten opercularis* and *Mimachlamys varia*. In: Shumway S. E., Parsons G. J. (Eds.) Scallops: biology, ecology and aquaculture. Amsterdam, Elsevier, pp. 991–1058.

Brookfield J.F., 1996, A simple new method for estimating null allele frequency from heterozygote deficiency. Mol. Ecol. 5, 453–455.

Cabranes C., Fernandez-Rueda P., Martinez J.L., 2008, Genetic structure of *Octopus vulgaris* around the Iberian Peninsula and Canary Islands as indicated by microsatellite DNA variation. ICES J. Mar. Sci. 65, 12–16.

Chambers G.K., MacAvoy E.S., 2000, Microsatellites: consensus and controversy. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 126, 455–476.

Chapuis M.P., Estoup A., 2007, Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24, 621–631.

Chistiakov D.A., Hellemans B., Volckaert F.A.M., 2006, Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255, 1–29.

Cragg S.M., Crisp D.J., 1991, The biology of scallop larvae. In: Shumway S. E. (Ed.) Scallops: Biology, Ecology and Aquaculture. Amsterdam, Elsevier, pp. 75–132.

Cruz F., Pérez M., Presa P., 2005, Distribution and abundance of microsatellites in the genome of bivalves. Gene 346, 241–247.

Diz A.P., Presa P., 2008, Regional patterns of microsatellite variation in *Mytilus galloprovincialis* from the Iberian Peninsula. Mar. Biol. 154, 277–286.

Edwards K.J., Barker J.H.A., Daly A., Jones C., Karp A., 1996, Microsatellite libraries enriched for several microsatellite sequences in plants. BioTechniques 20, 758.

Evans B.S., Knauer J., Taylor J.J.U., Jerry D.R., 2006, Development and characterization of six new microsatellite markers for the silver- or gold-lipped pearl oyster, *Pinctada maxima* (Pteriidae). Mol. Ecol. Notes 6, 835–837.

Excoffier L., Laval G., Schneider S., 2005, Arlequin version 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform, Online 1, 50.

Fernández-Moreno M., Arias-Pérez A., Freire R., Méndez J., 2008, Genetic analysis of *Aequipecten opercularis* and *Mimachlamys varia* (Bivalvia: Pectinidae) in several Atlantic and Mediterranean localities, revealed by mitochondrial PCR-RFLPs: a preliminary study. Aquac. Res. 39, 474–481.

Fernández-Tajes J., Méndez J., 2007, Identification of the razor clam species *Ensis arcuatus*, *E. siliqua*, *E. directus*, *E. macha*, and *Solen marginatus* using PCR-RFLP analysis of the 5S rDNA region. J. Agric. Food Chem. 55, 7278–7282.

Gaffney P.M., 1994, Heterosis and heterozygote deficiencies in marine bivalves: more light? In: Beaumont A. R. (Ed.) Genetics and evolution of aquatic organisms. London, Chapman & Hall, pp. 146–153.

Goudet J., 2001, FSTAT, a program to estimate and test gene diversities and fixation indices. Institut d'Ecologie, Université de Lausanne, Dorigny, Switzerland. http://www2.unil.ch/popgen/softwares/fstat.htm.

Hall T.A., 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Jensen J.L., Bohonak A.J., Kelley S.T., 2005, Isolation by distance, web service. BMC Genet. 6, 13.

Jørgensen H.B.H., Hansen M.M., Bekkevold D., Ruzzante D.E., Loeschcke V., 2005, Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol. Ecol. 14, 3219–3234.

Kenchington E.L., Patwary M.U., Zouros E., Bird C.J., 2006, Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). Mol. Ecol. 15, 1781–1796.

Launey S., Ledu C., Boudry P., Bonhomme F., Naciri-Graven Y., 2002, Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. J. Hered. 93, 331–351.

Lewis R.I., Thorpe J.P., 1994, Temporal stability of gene frequencies within genetically heterogeneous populations of the queen scallop *Aequipecten* (*Chlamys*) *opercularis*. Mar. Biol. 121, 117–126.

Li G., Hubert S., Bucklin K., Ribes V., Hedgecock D., 2003, Characterization of 79 microsatellite DNA markers in the Pacific oyster *Crassostrea gigas*. Mol. Ecol. Notes 3, 228–232.

Li Y.C., Korol A.B., Fahima T., Beiles A., Nevo E., 2002, Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Mol. Ecol. 11, 2453–2465.

Louis E.J., Dempster E.R., 1987, An exact test for Hardy-Weinberg and multiple alleles. Biometrics 43, 805–811.

Ma H., Yu Z., 2009, Development of twenty-two polymorphic microsatellite loci in the noble scallop, *Chlamys nobilis*. Conserv. Genet. 10, 1587–1590.

Macleod J.A.A., Thorpe J.P., Duggan N.A., 1985, A biochemical genetic study of population structure in queen scallop (*Chlamys opercularis*) stocks in the Northern Irish Sea. Mar. Biol. 87, 77–82.

Mathers N.F., 1975, Environmental variability at the phosphoglucose isomerase locus in the genus *Chlamys*. Biochem. Syst. Ecol. 3, 123–127.

Meglecz E., 2007, MICROFAMILY (version 1): a computer program for detecting flanking-region similarities among different microsatellite loci. Mol. Ecol. Notes 7, 18–20.

Nei M., 1978, Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.

Nève G., Meglécz E., 2000, Microsatellite frequencies in different taxa. Trends Ecol. Evol. 15, 376–377.

Quesada H., Zapata C., Alvarez G., 1995, A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. Mar. Ecol. Prog. Ser. 116, 99–115.

Raymond M., Rousset F., 1995, Genepop (Version-1.2): population-genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.

Reece K.S., Ribeiro W.L., Gaffney P.M., Carnegie R.B., Allen S.K., Jr, 2004, Microsatellite marker development and analysis in the eastern oyster (*Crassostrea virginica*): confirmation of null alleles and non-Mendelian segregation ratios. J. Hered. 95, 346–352.

Rice W.R., 1989, Analyzing tables of statistical tests. Evolution 43, 223–225.

Ríos C., Sanz S., Saavedra C., Pena J.B., 2002, Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia: Pectinidae), across the Almeria-Oran front. J. Exp. Mar. Biol. Ecol. 267, 223–244.

Rychlik W., Rhoads R.E., 1989, A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. Nucleic Acids Res. 17, 8543–8551.

Vadopalas B., Leclair L.L., Bentzen P., 2004, Microsatellite and allozyme analyses reveal few genetic differences among spatially distinct aggregations of geoduck clams *Panopea abrupta* (Conrad, 1849). J. Shellfish Res. 23, 693–706.

Van Oosterhout C., Hutchinson W.F., Wills D.P.M., Shipley P., 2004, MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.

Wagner H.P., 1991, Review of the European Pectinidae. Vita Marina 41, 1–48.

Was A., Gosling E., McCrann K., Mork J., 2008, Evidence for population structuring of blue whiting (*Micromesistius poutassou*) in the Northeast Atlantic. ICES J. Mar. Sci. 65, 216–225.

Watts P.C., Mallanaphy W.J., McCarthy C., Beukers-Stewart B.D., Mosley M.W.J., Brand A.R., Saccheri I.J., 2005, Polymorphic microsatellite loci isolated from the great scallop, *Pecten maximus* (Bivalvia: Pectinidae). Mol. Ecol. Notes 5, 902–904.

Weir B.S., Cockerham C.C., 1984, Estimating F-statistics for the analysis of population-structure. Evolution 38, 1358–1370.

Zane L., Bargelloni L., Patarnello T., 2002, Strategies for microsatellite isolation: a review. Mol. Ecol. 11, 1–16.

Zhan A., Bao Z., Hu X., Hui M., Wang M., Peng W., Zhao H., Hu J., 2007, Isolation and characterization of 150 novel microsatellite markers for Zhikong scallop (*Chlamys farreri*). Mol. Ecol. Notes 7, 1015–1022.

Zhan A., Hu J., Hu X., Hui M., Wang M., Peng W., Huang X., Wang S., Lu W., Sun C., Bao Z., 2009, Construction of microsatellite-based linkage maps and identification of size-related quantitative trait loci for Zhikong scallop (*Chlamys farreri*). Anim. Genet. 40, 821–831.