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Spatial Genetic Structure of the Surf Clam *Paphia undulata* in Thailand Waters

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Patipon Donrung, Suriyan Tunkijjanukij, Padermsak Jarayabhand, and Supawadee Poompuang (2011) Spatial genetic structure of the surf clam *Paphia undulata* in Thailand waters. *Zoological Studies* **50**(2): 211-219. The surf clam *Paphia undulata* has supported an offshore fishery in Thailand since the 1970s. However, most fishing sites have experienced declines in production over the past 2 decades. Overexploitation and low levels of genetic variation of surf clam populations may be responsible for the low productivity of the species. Inter-simple sequence repeat (ISSR) markers were used to assess the genetic diversity of surf clams sampled from 4 fishing areas in the Gulf of Thailand and 1 location in the Andaman Sea. In total, 300 ISSR loci were analyzed in 500 individuals. Three neighboring populations (SG, SS, and SP) in the upper Gulf of Thailand exhibited moderate genetic variation and similar Nei's gene diversity (H_j) values of 0.12-0.14, while populations from the lower Gulf of Thailand (SR) and the Andaman Sea (ST) had relatively low genetic variability with respective H_j values of 0.053 and 0.047. Different analyses, including F_{ST}, AMOVA, phylogenetic networks, and an assignment test revealed high levels of population substructuring, implying that gene flow may occur between stocks in the upper Gulf of Thailand, whereas the SR and ST populations were more geographically isolated. The relatively low amount of genetic diversity of the SR and ST stocks may reduce their ability to survive in a changing environment. Reductions of fishing efforts, and initiation of restocking programs and aquaculture development of this species should be considered to maintain the sustainability of the surf clam fishery. http://zoolstud.sinica.edu.tw/Journals/50.2/211.pdf

Key words: *Paphia undulata*, Genetic diversity, ISSR, Population genetics, Population structure.

The surf clam *Paphia undulata* (Mollusca: Bivalvia: Veneridae) is a commercially important bivalve distributed along the Gulf of Thailand and the Andaman Sea from the intertidal zone to a depth of 25 m (Sutthakorn and Tuaycharoen 1993). The surf clam has supported an offshore fishery in Thailand since the 1970s. Surf clams are recruited to the fishery at 2-3 cm in shell length at about 6-12 mo of age. The industry steadily grew for nearly 2 decades, from a production of 13,806 t in 1974 to 62,220 t in 1981 and to the highest yield of 131,230 t in 1987. However, since 1990, production has steadily declined to 31,495 t in 2002 (Department of Fisheries 2005). Populations at most of the fishing sites have deteriorated, and a year-round fishery is no longer available. Currently, surf clam fisheries are operated only in the provinces of Samut Songkram, Samut Sakorn, Samut Prakan, and Surat Thani on the Gulf of Thailand and in Satun Province on the Andaman Sea. Overexploitation, habitat degradation, and low genetic variability of surf clam populations could be major factors contributing to depletion of stocks. A restocking program was undertaken

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by the Department of Fisheries (DOF, Bangkok, Thailand) to recover the fishery. In 2002, approximately 10^6 surf clam larvae from an artificial breeding program were released into the eastern Gulf of Thailand in Trat Province (Department of Fisheries 2005). Unfortunately, the restocking was unsuccessful, and no evidence of the stock was found.

The future of the surf clam fishery; therefore, is highly dependent upon proper stock management of existing resources. It is widely recognized that genetic variation within populations affects the ability of a species to survive in a changing environment. For exploited stocks, assessment of genetic diversity and population structuring is a prerequisite for development of restocking programs and aquaculture in order to reduce fishing efforts (Ward 2006). Despite its commercial importance, genetic properties of surf clam populations have not been studied. The objectives of this study were to assess the genetic diversity and determine the genetic structuring of surf clam populations along the coasts of Thailand using genetic markers, thereby providing genetic information for management of fishery stocks and aquaculture.

Inter-simple sequence repeats (ISSRs) have become useful dominant markers for genetic investigations, particularly in a wide variety of plant species, due to their high polymorphism, technical simplicity, reproducibility, and cost-effectiveness (Zietkiewicz et al. 1994). The ISSR technique does not require genomic sequence information, and primers are designed from the core motifs of microsatellites. ISSR techniques are based on using a single primer that targets the repeat motif to amplify genomic regions between microsatellites. Applications of ISSR markers for population studies were reported for various species of mollusks including the amethyst gem clam *Gemma gemma* (Casu et al. 2005), the giant Mediterranean limpet *Patella furruginea* (Casu et al. 2006), the giant clam *Tridacna gigas* (Evans and Jerry 2006), and the Chinese surf clam *Mactra veneriformis* (Hou et al. 2006).

MATERIALS AND METHODS

Sample collection

In total, 500 surf clam samples were collected from 4 fishing sites in the Gulf of Thailand, including Samut Songkram (SG), Samut Sakorn (SS), Samut Prakan (SP), and Surat Thani (SR), and 1 location on the coast of the Andaman Sea (Satun, ST) (Fig. 1) by hydraulic clam dredge. The average size of surf clams in the samples was 2-3 cm in shell length, and they were about 6-12 mo old. Samples were placed in 95% ethanol until the DNA was extracted.

Fig. 1. Sample collection locations for *Paphia undulata* in the Gulf of Thailand and Andaman Sea. Abbreviations are given in table 2.

ISSR polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from the homogenized adductor muscle using a standard phenol-chloroform extraction procedure (Taggart 1992). Twenty ISSR primers (Wolf et al. 1998) synthesized by Ward Medic (Bangkok, Thailand) were tested on *P. undulata* DNA samples, nine of which were selected for genotyping. The PCR was performed in 20-μl reaction mixtures which contained 20 ng of template DNA, 1x PCR buffer (Fermentas, Vilnius, Lithuania), 0.25 μmol of each primer, 100 μmol/l of each dNTP, 1.5 μmol/l of $MgCl_2$, and 5 U of *Taq* polymerase (Fermentas). The PCR profile consisted of initial denaturation at 94°C for 5 min; then 40 cycles of 94°C for 1 min, annealing at temperatures given in table 1 for 1 min, and 72°C for 2 min; followed by 1 cycle at 72°C for 7 min in a PCT-100™ Programmable Thermal Controller (MJ Research, Waltham, MA, USA). A negative control was included with each set of PCRs. Following amplification, reaction products were mixed with loading buffer and subjected to electrophoresis through a 2% agarose gel at 100 V for 90 min. The gel was stained with ethidium bromide and visualized under ultraviolet light. The sizes of the amplified fragments were determined using a 100-bp DNA ladder (Fermentas).

Data analysis

ISSR bands were manually scored as present (1) or absent (0) based on their dominant mode of expression. Each band was considered to represent a single locus. Because it is not possible to distinguish homozygous dominant from heterozygous individuals with ISSR markers, several approaches were applied to calculate allelic frequencies. Within-population diversity statistics, including the percent polymorphic loci and Nei's gene diversity (H_j), were estimated by the Lynch and Milligan (1994) approach using AFLP SURV vers. 1.0 (Vekemans et al. 2002). The analysis was performed assuming Hardy-Weinberg equilibrium (HWE) (F_{IS} = 0), and low (F_{IS} = 0.05), moderate (F_{IS} = 0.10), or high (F_{IS} = 0.25) levels of inbreeding within populations. This approach was taken due to the frequency of heterozygote deficiencies in bivalves (Parker et al. 2003, Kenchington et al. 2006, Cassista and Hart 2007, Zhan et al. 2009). The values of diversity statistics increased only slightly with an increasing F_{IS}. Therefore, HWE was assumed for all populations. The significance of differences in average values of gene diversity (H_j) among populations was evaluated by a *t*-test using FSTAT vers. 2.9.3 (Goudet 2001).

The population genetic structure was assessed by calculating Nei's genetic distance and F_{ST} based on the Bayesian method with a uniform prior distribution of allelic frequencies using AFLP SURV vers. 1.0 (Vekemans et al. 2002). Population pairwise F_{ST} values were calculated and tested for significance by bootstrapping (1000 replicates) using TFPGA vers. 1.3 (Miller 1997). A phylogenetic network based on uncorrected P-distances was constructed using the neighbor-net algorithm implemented in SplitsTree4 vers. 4.11.3 (Huson and Bryant 2006), with 1000 replicates. The Structure 2.3.1 program (Pritchard et al. 2000) was used to determine levels of fine-scale structuring and to assign individuals into *K* populations without prior knowledge of population

Table 1. Inter-simple sequence repeat primer names, sequence (5'-3'), PCR annealing temperatures (T_A), total number of loci amplified, and size range of the amplified fragments

Primer	Sequence (5'-3')[a]	T_A (°C)	Total number of loci	Size range of fragments (bp)
ISSR3	$VBV(CA)_8$	54	40	200-1600
ISSR4	$VDV(GT)_8$	51	29	220-1400
ISSR9	$(AC)_8G$	55	28	180-940
ISSR10	$(TG)_8GT$	48	37	280-2200
ISSR11	$(AG)_8TG$	54	30	260-1400
ISSR12	$(TC)_8C$	52	27	280-1050
ISSR13	$(TG)_8G$	55	24	280-840
ISSR18	$(ACTG)_4$	52	42	220-1900
ISSR20	$(CAC)_6$	57	43	260-1800

[a]B = C, G, T; Y = C, T; R = A, G; H = A, C, T; V = A, C, G; D = A, G, T.

membership. Five independent runs for K = 1-5 were performed using 10^5 iterations after a burn-in period of 25,000 runs.

An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was performed to partition the total genetic variance into variance components distributed between: (1) groups of the Andaman sample and the pooled samples of the Gulf of Thailand and (2) Surat Thani and pooled samples of SG, SS, and ST, and to calculate the degree of differentiation within populations (Φ_{ST}) using FAMD (Schlüter and Harris 2006).

RESULTS

Genotyping of 500 surf clam samples from 5 locations using 9 ISSR primers yielded variations at 335 putative loci, of which 300 loci were analyzed following the method of Lynch and Milligan (1994), while 35 loci were excluded from further analyses of all populations due to low frequencies of the null phenotype (i.e., < 3 for a sample size of 100). The number of bands produced using each ISSR primer is shown in table 1. The number of total polymorphic loci in each population ranged from 40 (ST) to 141 (SG).

Three populations in the upper Gulf of Thailand exhibited moderate genetic variation and were similar in terms of percent polymorphic loci, which varied from 37.3% (SP) to 47.0% (SG), and Nei's gene diversity (H_j), which ranged from 0.12 (SP) to 0.14 (SG). Relatively low genetic variation was observed for the SR and ST populations, with 17.7% and 13.3% polymorphic loci and H_j values of 0.053 and 0.047, respectively (Table 2). The percent polymorphic loci and expected heterozygosities for the SR and ST populations significantly differed from each other and from the rest of the surf clam populations.

The overall estimate of F_{ST} of 0.315 (95% confidence interval: 0.313-0.316) indicated population differentiation. Seven of 10 pairwise F_{ST} comparisons exhibited significant differentiation ($p < 0.05$, Table 3). F_{ST} values indicated that 2 populations (SR and ST) significantly differed from each other and from all other populations (SG, SS, and SP). The split networks constructed for all 500 individuals revealed genetic relationships among these 5 populations of surf clam (Fig. 2). Clustering analysis in STRUCTURE identified 5 as the most likely number of clusters based on the highest average value of posterior probabilities. All individuals were correctly assigned to their respective population clusters. The proportions of membership of each population were high

Table 2. Percent polymorphic loci and Nei's gene diversity (H_j) obtained by Lynch and Milligan's (1994) method for inter-simple sequence repeat data for 5 populations of *Paphia undulata*

Population	Code	*n*	No. (percent) of polymorphic loci	H_j
Samut Songkram	SG	100	141 (47.0%)	0.141 ± 0.008
Samut Sakorn	SS	100	137 (45.7%)	0.128 ± 0.007
Samut Prakan	SP	100	112 (37.3%)	0.122 ± 0.009
Surat Thani	SR	100	53 (17.7%)	0.053 ± 0.006
Satun	ST	100	40 (13.3%)	0.047 ± 0.006

Table 3. Estimates of pairwise F_{ST} values (above the diagonal) and Nei's genetic distance (below the diagonal) based on inter-simple sequence repeat data for 5 populations of *Paphia undulata* in Thailand

Population[a]	SG	SS	SP	SR	ST
SG	-	0.179	0.199	0.299*	0.348*
SS	0.033	-	0.202	0.346*	0.372*
SP	0.039	0.037	-	0.338*	0.360*
SR	0.050	0.057	0.054	-	0.485*
ST	0.063	0.064	0.060	0.060	-

*An asterisk denotes significant population differentiation ($p < 0.05$) after applying the sequential Bonferroni correction. [a]Population codes are given in table 2.

with respective values of 0.963, 0.963, 0.979, 0.994, and 0.994 for the SG, SS, SP, SR, and ST populations (Table 4).

Results of the AMOVA based on Jaccard's similarity coefficient demonstrated that significant variation was distributed between groups of pooled samples in the Gulf of Thailand and Andaman Sea (53.28%) and within populations (40.37%), while variance among populations within groups was relatively small (6.35%). The Φ_{ST} value of 0.596 ($p < 0.001$) also suggested a very high level of genetic differentiation among populations (Table 5). Higher variation was observed between the ST population and pooled samples from SG, SS, and SP (68.9%), with a Φ_{ST} value of 0.734 ($p < 0.001$).

DISCUSSION

Marine species are generally believed to possess high levels of within-population genetic diversity, but to display weak between-population structuring. This is due to their large population sizes, high fecundity, and extensive gene flow caused by the high dispersal of adults, larvae, and gametes, as well as by a lack of barriers in the open seas (Waples 1998). In addition, many studies suggested that factors such as ocean currents can facilitate gene flow over large geographical distances. In marine invertebrates, gene flow normally occurs through the high dispersal ability of planktonic larvae, because adults are typically sessile. Species with a long planktonic larval stage; therefore, tend to be more homogeneous. However, this perception has been challenged, as an increasing number of studies have found evidence of population substructuring in marine bivalves over either small or large geographical scales (Casu et al. 2005, Evans and Jerry 2006, Kenchington et al. 2006, Lind et al. 2007, Zhan et al. 2008). The presence of population substructuring was also reported in *Octopus vulgaris* (Casu et al. 2002, Maltagliati et al. 2002).

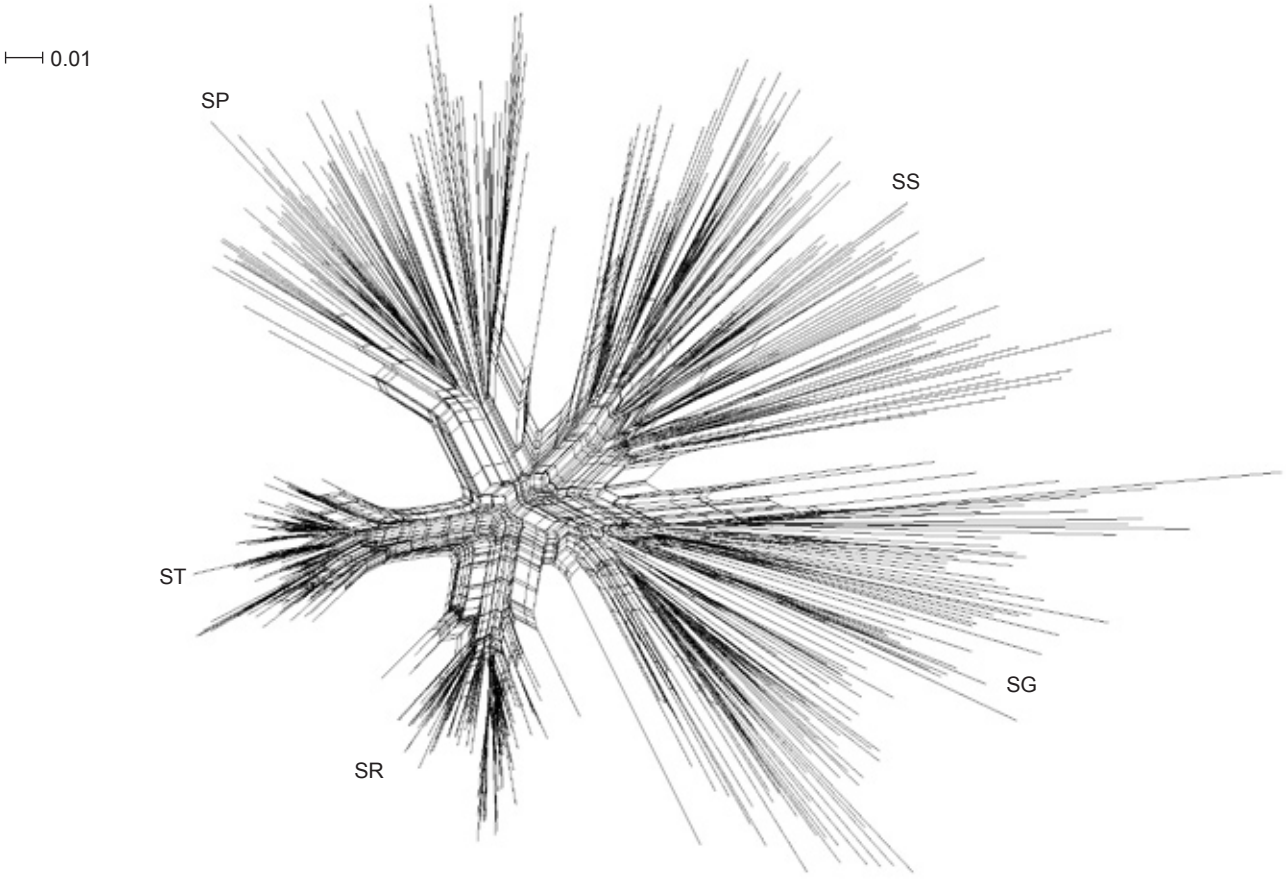

Fig. 2. Neighbor network constructed from 500 individuals displaying grouping of 5 populations of *Paphia undulata*. Abbreviations are given in table 2.

The commercially exploited stocks of surf clam in Thailand exhibited moderate to low levels of genetic variability and strong population substructuring as revealed by ISSR markers. Overall, neighboring populations from the upper Gulf of Thailand, including SG, SS, and SP, were similar, but markedly differed from the SR sample in the lower Gulf of Thailand and the single stock from the Andaman Sea (ST). The similarity of these neighboring stocks suggests substantial gene flow between populations. Moreover, the presence of moderate genetic diversity in these locations may be attributable to the historic effects of a large effective population size and to the contemporary effect of a reserve area in Samut Sakorn Province, where fishing is prohibited. Marine protected areas particularly focus on conservation and enhancement of exploited stocks. The reserve area was established in 2002 (Department of Fisheries 2005). In contrast, the geographical isolation of the SR and ST populations coupled with the absence of gene flow could have resulted in lower diversity within these populations. Across populations, differences in the genetic makeup of surf clams were likely due to the effects of clumped sampling and a small effective population size. Because sampling was not continuous throughout the geographical range of the study area, some fraction of the variance may be attributed to the clumped sampling. Additionally, high variation in the reproductive success of breeders may have limited the effective size of populations. This characteristic was noted in species with high fecundity and a high rate of early mortality such as bivalves and shrimp (Li and Hedgecock 1998, Ball and Chapman 2003). Intense fishing efforts for more than 3 decades may have contributed to reduced effective population sizes of surf clams. Overfishing was reported to be a major factor causing a significant reduction in the effective population size and reduced genetic diversity in an exploited stock of New Zealand snapper *Pagrus auratus* (Hauser et al. 2002). The distinct differentiation between the Andaman Sea and Gulf of Thailand samples was likely due to the geographical barrier caused by the Malaysian Peninsula. This finding is concordant with reports on abalone *Haliotis asinina* (Tang et al. 2005), the Asian moon scallop *Amusium pleuronectes* (Mahidol et al. 2007), and spotted seahorse *Hippocampus kuda* (Panithanarak et al. 2010).

Populations of the surf clam *P. undulata* in this study seemed to possess relatively low diversity (H_j = 0.047-0.14) compared to that of the Chinese surf clam *M. veneriformis* (H_j = 0.212-0.342) (Hou et al. 2006). Nevertheless, the ISSR diversity of *P. undulata* was in the same range as those of *G. gemma* (H_j = 0.169-0.184; Casu et al. 2005) and

Table 4. Proportion of membership of each predefined *Paphia undulata* population in each of the 5 inferred data clusters

Population[a]	Inferred cluster				
	1	2	3	4	5
SG	0.963	0.007	0.007	0.017	0.006
SP	0.003	0.006	0.979	0.004	0.007
SR	0.002	0.002	0.001	0.994	0.001
SS	0.018	0.008	0.005	0.006	0.963
ST	0.001	0.994	0.001	0.003	0.001

[a]Population codes are given in table 2.

Table 5. Analysis of molecular variance (AMOVA) of *Paphia undulata* using the FAMD program: (1) between the Andaman Sea and pooled samples of the Gulf of Thailand, and (2) between Surat Thani and pooled samples of Samut Songkram, Samut Sakorn, and Samut Prakan

	Source of variation	*d.f.*	Sum of squares	Variance components	Percent variation	Φ_{ST}
(1)	Between groups	1	22.52	0.246	53.29	-
	Among populations within groups	3	74.42	0.034	6.34	-
	Within populations	495	82.73	0.167	40.37	0.596
	Total	499	179.68	0.447		
(2)	Between groups	1	83.12	0.414	68.98	-
	Among populations within groups	2	63.12	0.044	4.56	-
	Within populations	396	74.04	0.148	26.46	0.735
	Total	399	220.28	0.607		

Patella ferruginea (H_j = 0.173-0.233; Casu et al. 2006). Previous studies with allozymes suggested a moderate amount of genetic variation in surf clams of a different genus, the Australian surf clam *Donax serra* (42%-75% polymorphic loci; Murray-Jones and Ayre 1997) and African surf clam *D. deltoids* (18%-22% heterozygosity; Laudien et al. 2003). In another study, a microsatellite analysis revealed high levels of within-population genetic diversity in the Arctic surf clam *Mactromeris polynyma* (Cassista and Hart 2007). However, it should be noted that estimates of genetic diversity may differ among species and between marker systems.

The population structure of *P. undulata* was strong (overall F_{ST} = 0.315) and comparable to that of *M. veneriformis* (F_{ST} = 0.202; Hou et al. 2006). The F_{ST} metric is a measure of the reduction in heterozygosity in a species due to population structuring. In this study, a subdivision of populations, possibly due to natural history, genetic drift, and selection, accounted for approximately 31% of the total genetic variation. Genetic differentiation among populations was also reflected by pairwise F_{ST} calculations, of which 70% significantly differed from each other. AMOVA results indicated that approximately 53% of the variance occurred between populations of the Andaman Sea and Gulf of Thailand.

Differences among surf clam populations within a small geographical range of approximately 450 km in the Gulf of Thailand were also attributed to limited gene flow. Surf clam larvae spend about 12 d as plankton followed by metamorphosis and a sedentary phase (Pongthana 1988). A number of studies indicated that ocean currents or marine landscapes can either facilitate or prevent gene flow over large geographic scales (Hellberg 2009). For instance, a broadcast-spawning species, such as the sea scallop *P. magellanicus* in the North Atlantic Ocean of Canada, exhibited significant population differentiation, despite having an approximately 30-d planktonic phase (Kenchington et al. 2006). Similarly, the pearl oyster *P. maxima* (with a 17-24 d larval phase) showed strong population structuring over the Indonesian-Australian region (Lind et al. 2007). However, genetic homogeneity over broad geographic scales was reported in the Australian surf clam (Murray-Jones and Ayre 1997), African surf clam (Laudien et al. 2003), and Arctic surf clam (Cassista and Hart 2007). A high dispersal ability due to a long larval phase (30-60 d) and ocean currents were major factors in the lack of genetic structuring in those populations.

The analysis of phylogenetic networks supported the characterization of surf clam population substructuring into 5 distinct groups. The existence of significant population structuring of surf clams in Thailand was further demonstrated by the assignment test, in which individuals were assigned to their respective populations with high proportions of membership. The test clearly separated the SG and SS populations. Despite a very short distance between sites (35 km), the 2 populations did not appear to be connected, and these populations were not connected with the closer group of SP. It was also suggested that even though the populations are rather isolated, they have only been separated for a short period of time as observed by small estimates of genetic distances.

The relatively low amount of genetic diversity of the SR and ST populations may reduce their ability to survive in a changing environment. Therefore, conservation and management plans should be undertaken to maintain genetic variation of these isolated and differentiated populations. Fishing efforts should be more restricted to allow recovery of stocks. For restocking programs, broodstock should be collected from local populations to avoid genetic contamination and benefit from local adaptations (Miller and Kapuscinski 2003). Assessing temporal genetic variations may be useful for estimating effective population sizes. Aquaculture of surf clams should be established to meet increased demands for production.

This study showed that ISSR markers are useful for revealing fine-scale population structuring of marine bivalves. However, investigations using other types of markers such as microsatellites should be conducted to test the assumption of random mating and to estimate the effective sizes of surf clam populations. In addition, sampling in areas between the studied sites should be conducted to further test migration and population structuring, as well as sampling throughout the range of this species to identify any discontinuities in gene flow.

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