Biogeography **19.**
61–68. Sep. 20, 2017

Development of compound polymorphic microsatellite markers for the pronghorn spiny lobster *Panulirus penicillatus* and comparison of microsatellite data with those of a previous mitochondrial DNA study performed in the northwestern Pacific

Muhamad Fadry Abdullah[1,4], Jin-Hua Cheng[2], Tzyy-Ing Chen[2] and Hideyuki Imai[3,*]

[1] Marine and Environmental Sciences, Graduate School of Engineering and Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan
[2] Tungkang Biotechnology Research Center, Taiwan Fisheries Research Institute, Council of Agriculture, Tungkang, Pingtung 928, Taiwan
[3] Laboratory of Marine Biology & Coral Reef Studies, Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan
[4] Present Address: PT. Indonesia Kyouei Saikyu, Jl. Harapan1 Lot KK-2C KllC, Sivnabaya, Telukjambe Timur, Kabupaten Karawang, Jawa Barat 41361, Indonesia

Abstract. Ten novel di- and tri-nucleotide polymorphic microsatellite loci were identified to aid in genetic structure analysis of the widely distributed pronghorn spiny lobster, *Panulirus penicillatus*. Genetic variation among populations was explored, and we compared our results to those of a previous mitochondrial DNA (mtDNA) analysis (Abdullah et al. 2014) conducted in the northwestern Pacific Ocean. A total of 129 *P. penicillatus* individuals were collected from three localities in Japan (Hachijojima Island, Okinawajima Island and Ishigakijima Island) and one in Taiwan (Taitung). F-statistical analysis revealed no significant genetic differences among animals collected at the different localities, in agreement with data from the previous mtDNA study. The combined data suggest that high-level gene flow exists among the explored localities, probably caused by the extended planktonic larval stage and the prevailing Kuroshio Current. On the scale of the northwestern Pacific, the results obtained in both studies suggest that *P. penicillatus* forms a single unique panmictic population, or at least that the several breeding grounds exchange significant amounts of genetic material.
Key words: *Panulirus penicillatus*, genetic diversity, population structure, microsatellite DNA

Introduction

Of the spiny lobsters, the pronghorn *Panulirus penicillatus* (Olivier 1811) probably has the widest distribution. The lobster is found in tropical and adjacent regions ranging from south-eastern Africa, the Red Sea, southern India, the Southeast Asian archipelago, Japan, northern Australia, and the southern and western Pacific islands to Hawaii, the Galápagos Islands, and other islands of the eastern Pacific (Holthuis 1991, Pitcher 1993). *P. penicillatus* supports several large fisheries in tropical Pacific regions. The lobster harvest is economically and socially important to the inhabitants of Pacific island nations. Although the lobster is a valuable marine resource for many countries, the genetic diversity and population structure of the species remain poorly known (Abdullah et al. 2014). Microsatellite markers would aid in the study of the detailed genetic structures of *P. penicillatus* populations. We developed simple sequence repeat (SSR) markers using an adaptor-ligation technique (Lian et al. 2006) and compared our results with those of a previous mtDNA analysis (Abdullah et al. 2014) to explore

*Corresponding author: imai@sci.u-ryukyu.ac.jp

the genetic structure of the pronghorn spiny lobster *P. penicillatus* in the northwestern Pacific Ocean.

MATERIALS AND METHODS

Microsatellite-Enriched Library Construction and Isolation of Microsatellite Markers

Total genomic DNA was extracted from lobster tissue using a phenol-chloroform procedure and precipitated with absolute ethanol, as described by Imai et al. (2004). A restricted adaptor-ligated DNA library was constructed as described by Lian et al. (2006). DNA was digested separately using *Alu*I, *Afa*I, *Eco*RV, *Sau*3AI, and *Ssp*I, and the resulting fragments were ligated at the 5′ ends to a blunt 48-mer adaptor (5′-GTAATACGACTCACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGGT-3′) and at the 3′ ends to an 8-mer capped by an amino residue (5′-ACCAGCCC-NH2-3′), using a DNA ligation kit (Takara Bio). To enrich microsatellite regions, fragments flanked by a microsatellite at one end were amplified from the library using compound SSR primers, $(AC)_6(AG)_5$, $(TC)_6(AC)_5$, $(AGC)_4(AGG)_3$, or $(TTG)_4(TGG)_3$, and an adaptor primer, AP2 (5′-CTATAGGGCACGCGTGGT-3′), designed from the long strand of the adaptor. The amplified fragments were subcloned using the pT7 Blue Perfectly Blunt Cloning Kit (Novagen). Recombinant clones were identified via blue/white screening on LB agar plates containing ampicillin, X-gal, and isopropyl-β-D- thiogalactopyranoside (IPTG). The cloned fragments were amplified from plasmid DNAs containing the positive clones using U19 and M13 reverse primers, and the fragment lengths were verified via 1% (w/v) Trevigel Powder gel electrophoresis (Trevigen). The amplified fragments were sequenced on an ABI 3730xl Genetic Analyzer (Applied Biosystems). For each fragment that contained compound sequences at one end, a specific primer (SP) was designed from the sequenced region flanking the microsatellite. Each primer pair (SP and the corresponding compound SSR primer) served as a compound microsatellite marker. PCR primers were designed using Primer3 (Rozen and Skaletsky 2000).

Genotyping and Data Analysis

We analyzed SSR polymorphisms in adult 129 individuals of *P. penicillatus* collected from three localities in Japan (Hachijojima Island, Okinawajima Island and Ishigakijima Island) and one in Taiwan (Taitung) (Table 1). Polymerase chain reactions were performed in a 25-μL reaction volume containing 1 μL total genomic DNA, 12.5 μL AmpliTaq Gold® 360 Mastermix (Applied Biosystems), 0.4 μL (25 pmol/μL) each primer, 1.8 μL 360 GC Enhancer, and 8.5 μL nuclease-free water. Amplification was performed using the aid of a GeneAmp PCR System 9700 (Perkin Elmer) under the following conditions: initial denaturation at 95°C for 1 min; 39 cycles of denaturation at 95°C for 45 s, annealing at a primer-dependent temperature for 45 s, and extension at 72°C for 45 s; and a final extension at 72°C for 5 min. Ten of the 48 primer pairs yielded useful information, while the remaining 38 did not; either genetic variation was low or no amplicon was noted. The characteristics of 10 microsatellites are shown in Table 2.

For genotyping, PCR amplification was performed as described above using forward primers fluorescently labeled with Beckman Dye D3 or D4 (Sigma-Aldrich). Genotypes were scored using the CEQ 8800 Genetic Analysis System (Beckman Coulter). PCR products were diluted in 100 μL distilled water, and 1 μL of each of these templates was mixed with 0.1 μL GenomeLab DNA Size Standard 400 (Beckman Coulter) and 30 μL GenomeLab™ Sample

Table 1. Sample localities, locality IDs, numbers of samples (n), and the years in which the *Panulirus penicillatus* samples subjected to microsatellite analysis were collected.

Locality	n	Year	Sample ID
Hachijojima Island, Japan	33	2009	HC
Okinawajima Island, Japan	33	2008	OK
Ishigakijima Island, Japan	30	2008	IG
Taitung, Taiwan	33	2009	TW

Loading Solution (containing formamide) (Beckman Coulter). CEQ 8800 software version 9.0.25 was used to determine the sizes of the microsatellite alleles.

Expected (*He*) and observed (*Ho*) (Nei 1978) heterozygosity estimates for each locus across all localities were calculated using the Excel Microsatellite Toolkit (Park 2001). The number of alleles per locus (a), the extents of Hardy-Weinberg equilibrium (HWE), and pairwiseΦ_{ST} values were estimated using Arlequin version 3.5 (Excoffier et al. 2005). Allelic richness values (ar) and inbreeding coefficients (F*is*) were calculated using FSTAT version 2.9.3.2 (Goudet, 1995). The null-allele frequencies of all 10 loci across all localities were estimated using MICRO-CHECKER (Van Oosterhout et al. 2004). BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996, Piry et al. 1999) was used to evaluate the theoretical prediction that a population bottleneck would trigger a faster reduction in allelic diversity than would heterozygosity, in turn generating an excess of heterozygotes in the post-bottleneck population. We used the two-phase model (TPM) of mutation recommended for analysis of microsatellite loci; this model better fitted the observed allelic frequency data than did the infinite alleles model (IAM) or the single-step model (SSM) (Di Rienzo et al. 1994). The Bayesian model-based clustering program STRUCTURE version 2.3.4 (Pritchard et al., 2000; Falush et al., 2003) was employed to detect species-level structures and to assign individuals to groups. This method uses multi-locus genotypes and a predefined number of clusters (K) to generate groupings minimizing deviation from HWE and linkage equilibrium. Individuals are probabilistically assigned to one or more clusters based on their multi-locus genotypes. To estimate past migration rates and to assess the potential for asymmetric gene flow between localities, we used the coalescence-based approach implemented in MIGRATE-N version 3.6.4 (Beerli and Felsenstein, 1999, 2001). This software employs a maximum-likelihood method or Bayesian inference to estimate the parameter M ($M = m/u$, where m is the migration rate and u the mutation rate), which in turn gives the mutation-scaled long-term migration rate between localities. To compare

Table 2. *Panulirus penicillatus* microsatellite loci showing locus IDs, the sequences of forward (F) and reverse (R) primers, repeat motifs, annealing temperatures (°C), and accession numbers.

Locus ID	Primer sequence (5'-3')	Repeat motif	T_a (°C)	Accession number
PP-AC07	F: ACACACACACACAGAGAGAGAG R: GGTCCTCGGGAGATTAGGTC	$(AC)_6(AG)_{12}$	57	AB901193
PP-AC4e	F: ACACACACACACAGAGAGAGAG R: AAACCATAGTCTTGCATGCTGGT	$(AC)_6(AG)_7$	61	AB901194
PP-TC1a	F: TCTCTCTCTCTCACACACACAC R: AGATTATCCCCTTCACGCCTATC	$(TC)_6(AC)_6$	61.5	AB901195
PP-TC10c	F: TCTCTCTCTCTCACACACACAC R: GTTAAGCCTCATGCGTCATTCT	$(TC)_6(AC)_9$	59.5	AB901196
PP-AGC1a	F: AGCAGCAGCAGCAGGAGGAGG R: GGAGGCGTTGGTGTAAGAGGT	$(AGC)_4(AGG)_4$	61.5	AB901197
PP-AGC3b	F: AGCAGCAGCAGCAGGAGGAGG R: GCTACTCGCCACTCACCACAG	$(AGC)_4(AGG)_5$	61.5	AB901198
PP-AGC4a	F: AGCAGCAGCAGCAGGAGGAGG R: TTTTGCGCCCCTGAACAAATT	$(AGC)_1(AGG)_7$	55.5	AB901199
PP-AGC4e	F: AGCAGCAGCAGCAGGAGGAGG R: CGCGGTTTCTACCAGTCTCTTTT	$(AGC)_4(AGG)_6$	61	AB901200
PP-AGC13e	F: AGCAGCAGCAGCAGGAGGAGG R: GAGGCGTTGGTGTAAGAGGTG	$(AGC)_4(AGG)_3$	61	AB901201
PP-TTG4d	F: TTGTTGTTGTTGTGGTGGTGG R: CTACGGGAAGGTAGGGAACGTA	$(TTG)_4(TGG)_4$	59	AB901202

our results with earlier mtDNA data (Abdullah et al. 2014), we entered control sequences from Abdullah et al. (2014) into a DNA sequence model parameter running on MIGRATE-N version 3.6.4.

RESULTS AND DISCUSSION

In total, 48 primer pairs were designed, of which 10 were both polymorphic and co-dominant. The remaining 38 were not useful; either the level of genetic variation was low or no amplicon was obtained. Genetic variability estimates for *P. penicillatus* at the four localities are shown in Table 3. The microsatellite loci used were highly polymorphic, although the extent of variability differed among loci. The number of observed alleles at each locus ranged from 2 to 26 (average 8.8), and the observed and expected heterozygosities ranged from 0.091 to 0.893 and 0.391 to 0.961, respectively. MICRO-CHECKER predicted the existence of null alleles at six loci. The predicted null estimates (Chakraborty calculation) were 0.057, 0.26, 0.07, 0.58, 0.53, and 0.52 for the loci *PP-AC07*, *PP-TC1A*, *PP-TC10C, PP-AGC4A*, *PP-AGC4E*, and *PP-TTG4D*, respectively. The presence of null alleles was not surprising, as these are commonly observed in abundant marine invertebrates (Kaukinen et al. 2004, Ben-Horin et al. 2009). Null alleles were not detected in the remaining loci. Deviations from HWE were evident in some loci (Table 3). Heterozygote deficiencies among marine invertebrates are common (Maggioni and Rogers 2002, Damodaran et al. 2008) and have indeed been observed in other lobsters [*P. cygnus* (Groth et al. 2009) and *P. interruptus* (Ben-Horin et al. 2009)]. A deviation from HWE may be caused by the presence of null alleles, population subdivisions, the Wahlund effect, and/or inbreeding.

We applied the TPM to determine whether the microsatellite data could yield evidence of a recent bottleneck. A significant departure from mutation-drift equilibrium was evident (Wilcoxon test; $P<0.05$) (Table 3), suggesting that the population experienced a recent bottleneck that reduced genetic diversity. Although the deviations from HWE evident in several localities may have biased the results, the positive bottleneck tests likely reflect fluctuations in population sizes.

The pairwise Φ_{ST} estimates ranged from 0.00359 (between Okinawajima Island and Taitung, Taiwan) to 0.01294 (between Ishigakijima and Okinawajima Island) (Table 4). The pairwise Φ_{ST} P-values did not differ significantly for any comparison. F-statistics revealed the absence of any significant genetic difference among localities, in agreement with data from a previous study on mtDNA. STRUCTURE found no boundaries among subpopulations at K=2, 3, or 4. Thus, mixing clearly occurred among lobster located at all four localities evaluated in the northwest Pacific (Fig. 1).

MIGRATE-N version 3.6.4 was used to examine gene flow patterns among individual lobsters of the northwest Pacific. Both nuclear and mitochondrial data were analyzed, first, using a Brownian motion configuration to examine microsatellite data and, second, using control sequences from Abdullah et al. (2014). The latter analysis was configured using DNA sequence model parameters and was run on MIGRATE-N version 3.6.4. Migration rates between individual localities varied by several orders of magnitude in either analysis, in line with the calculated Φ_{ST} values. Migration rates between localities were generally asymmetrical; migration from the direction of the Kuroshio Current was typically greater than migration in the reverse direction (Fig. 2). MIGRATE-N also detected relatively high rates of gene flow from higher to lower latitudes, suggesting the existence of a countercurrent effect, which supports an earlier suggestion that during an earlier evolutionary period, *P. japonicus* inhabited our study area (Sekiguchi and Inoue 2002). Additionally, Chow et al. (2006) found that mid-to-final stage phyllosoma larvae were relatively abundant south of Ryukyu Archipelago (see rectangularly shaded areas in Fig. 2). Migration rate analysis showed that regional *P. penicillatus* constituted a meta-population, featur-

Table 3. Genetic variability estimates of 10 microsatellite loci from *Panulirus penicillatus*.

Locality	F*is*	*P*§		Locus										Mean of all loci
				PP-AC07	PP-AC4e	PP-TC1a	PP-TC10c	PP-AGC1a	PP-AGC3b	PP-AGC4a	PP-AGC4e	PP-AGC13e	PP-TTG4d	
Hachijojima, Japan	0.215	0.0161		#		##	#			##	##	#	##	
			a	9	4	23	21	5	2	8	8	5	4	8.9
			ar	8.2	4.0	22.2	20.0	4.8	2.0	7.8	8.0	4.7	3.8	8.6
			ho	0.606	0.576	0.813	0.848	0.485	0.375	0.242	0.364	0.697	0.182	0.519
			he	0.759	0.539	0.956	0.950	0.441	0.437	0.811	0.754	0.519	0.424	0.659
			ho/he	0.799	1.068	0.850	0.893	1.098	0.859	0.299	0.482	1.342	0.429	0.812
Okinawajima, Japan	0.391	0.0093			##	##	##	##	##	##	##	#	##	
			a	6	6	26	17	6	3	6	8	6	5	8.9
			ar	5.7	5.8	24.3	16.3	5.8	3.0	6.0	7.8	5.8	4.7	8.5
			ho	0.667	0.667	0.394	0.758	0.424	0.333	0.152	0.303	0.394	0.091	0.418
			he	0.711	0.679	0.955	0.934	0.585	0.517	0.799	0.629	0.451	0.567	0.683
			ho/he	0.937	0.982	0.413	0.811	0.726	0.645	0.190	0.481	0.874	0.160	0.622
Ishigakijima, Japan	0.244	0.0010		##	##		#			##	##		##	
			a	8	4	24	19	5	2	8	5	6	3	8.4
			ar	7.9	4.0	23.8	19.0	5.0	2.0	8.0	5.0	5.9	3.0	8.4
			ho	0.633	0.700	0.448	0.893	0.786	0.621	0.138	0.100	0.633	0.133	0.509
			he	0.786	0.588	0.961	0.936	0.603	0.494	0.742	0.642	0.560	0.391	0.670
			ho/he	0.805	1.191	0.467	0.954	1.302	1.257	0.186	0.156	1.131	0.341	0.779
Taitung, Taiwan	0.262	0.0024			#	##	##			#	##		##	
			a	7	6	26	19	6	2	7	7	6	3	8.9
			ar	6.7	5.7	25.4	18.6	5.8	2.0	6.8	7.0	5.7	3.0	8.7
			ho	0.697	0.758	0.600	0.767	0.710	0.300	0.303	0.061	0.606	0.212	0.501
			he	0.681	0.696	0.959	0.933	0.567	0.413	0.765	0.677	0.531	0.541	0.676
			ho/he	1.023	1.089	0.625	0.822	1.252	0.726	0.396	0.089	1.140	0.392	0.756
Mean of all Localities			a	7.5	5.0	24.8	19.0	5.5	2.3	7.3	7.0	5.8	3.8	8.8
			ar	7.1	4.9	23.9	18.5	5.4	2.3	7.2	6.9	5.5	3.6	8.5
			ho	0.651	0.675	0.564	0.816	0.601	0.407	0.209	0.207	0.583	0.155	0.487
			he	0.734	0.625	0.958	0.938	0.549	0.465	0.779	0.676	0.515	0.481	0.672
			ho/he	0.891	1.083	0.589	0.870	1.095	0.872	0.268	0.302	1.122	0.331	0.742

F*is*: inbreeding coefficient, §: One tailed *P* value for Wilcoxon's test of heterozygosity excess under T.P.M
a: No. of allele, ar: Alleic richness, ho: Heterozygosity observed he:Heterozygosity estimated
#: Departure from Hardy-Weinberg equiribrium (#: $P<0.05$, ##: $P<0.001$)

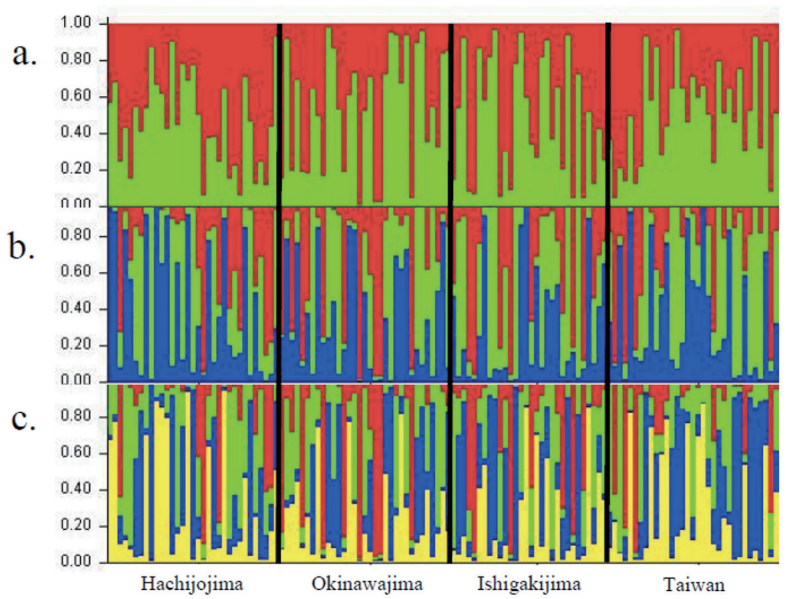

Fig. 1. Population assignments. (a) K = 2, (b) K = 3, and (c) K = 4, derived using STRUCTURE to analyze 10 microsatellite loci of *Panulirus penicillatus*.

Table 4. Pairwise Φ_{ST} values (above the diagonal) and Φ_{ST} *P-values* (below the diagonal) of microsatellite loci among the localities at which *Panulirus penicillatus* was found.

Localities	Hachijojima, Japan	Okinawajima, Japan	Ishigakijima, Japan	Taitung, Taiwan
Hachijojima, Japan		0.01241	0.00917	0.00377
Okinawajima, Japan	0.05957		0.01294	0.00359
Ishigakijima, Japan	0.10742	0.08105		0.00841
Taitung, Taiwan	0.44629	0.56836	0.16895	

ing extensive exchange of genetic material among component populations. Thus, high genetic diversity was evident within the localities evaluated, but little genetic differentiation was seen among the localities.

The microsatellite data combined with the mitochondrial DNA data of Abdullah et al. (2014) suggest high gene flow among the localities evaluated, probably because of the extended planktonic larval stage and the prevailing Kuroshio Current. On the scale of the northwestern Pacific, the results obtained using either marker are consistent with the notion that *P. penicillatus* forms a single unique panmictic population or, at least, that populations of several breeding grounds exchange significant amounts of genetic material.

Acknowledgements

We would like to thank to all the members of Imai-Lab for advice and technical support. This research was supported by the Okinawa Prefectural Government (the Life History and Genetic Population Structure for Important Fisheries Fishes). This research was partially supported by the JSPS KAKENHI (Grant-in-Aid for Scientific Research C) JP17K07918 and Okinawa Research Core for Highly Innovative Discipline Science (ORCHIDS) project, University of the Ryukyus.

References

Abdullah, M. F., Chow, S., Sakai, M., Cheng, J. -H., Imai, H. 2014. Genetic diversity and population structure of pronghorn spiny lobster *Panulirus*

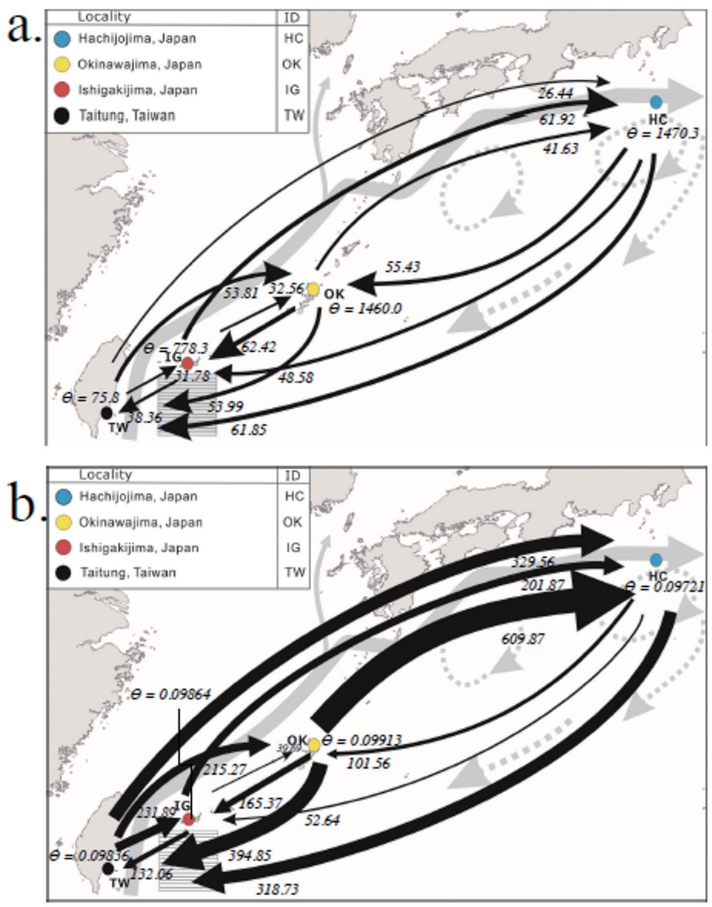

Fig. 2. Bayesian inference estimates of long-term migration rates (M = black arrows) and mutation-scaled effective population sizes (Θ values) in northwest Pacific populations of *Panulirus penicillatus*, determined using (a) microsatellite loci (data of the present study) and (b) mtDNA data (Abdullah et al. 2014) employing MIGRATE-N. The thickness of the shafts of the black arrows indicate directional migration rates ($M = m/u$, where m is the migration rate and u the mutation rate). Gray arrows indicate the Kuroshio Current (unbroken line) and the Kuroshio Countercurrent (dashed line) (Kawai 1991). Shaded rectangular areas indicate the locations of *P. penicillatus* phyllosoma larva sightings in Ryukyu Archipelago by Chow et al. (2006).

penicillatus in the Pacific region. *Pacific Science*, **68**: 197-211.

Beerli, P., Felsenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences of USA*, **98**: 4563-4568.

Ben-Horin, T., Lacchei. M., Selkoe, K. A., Mai, T. T., Toonen, R. J. 2009. Characerization of eight polymorphic microsatellite loci for the California spiny lobster, *Panulirus interruptus* and crossamplification in other achelate lobsters. *Conservation Genetics Resources*, **1**: 193-197.

Chow, S., Yamada, H., Suzuki, N. 2006. Identification of mid-to final stage phyllosoma larvae of the genus *Panulirus* White 1847 collected in the Ryukyu Archipelago. *Crustaceana*, **79**: 745-764.

Cornuet, J. M., Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**: 2001-2014.

Damodaran, D., Karunasagar, I., Karunasagar, I. 2008. Microsatellite DNA markers in the giant freshwater prawn, *Macrobrachium rogenbergii*: a

tool for genetic analysis. *Molecular Ecology Resources*, **8**: 1040-1042.

Di Rienzo, A., Peterson, A. C., Garza, J. C., Valdes, A. M., Slatkin, M., Freimer, N. B. 1994. Mutational processes of simple-sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences*, **91**: 3166-3170.

Excoffier, L., Laval, G., Schneider, S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**: 47-50.

Falush, D., Stephens, M., Pritchard, J. K. 2003. Inference of population structure using multilocus genetype data: linked loci and correlated allele frequencies. *Genetics*, **164**: 1567-1587.

Goudet, J. 1995. FSTAT (version 1.2): A computer program to calculate F-statistics. *Journal of Heredity*, **86**: 485-486.

Groth, D. M., Lim, F., de Lestang, S. N., Beale, N., Melville-Smith, R. 2009. Characterization of polymorphic microsatellite loci in the western rock lobster (*Panulirus cygnus*). *Conservation Genetics Resources*, **1**: 163-166.

Holthuis, L. B. 1991. *Marine Lobsters of the World: an annotated and illustrated catalogue of species of interest to fisheries known to date*. FAO Fish Synopsis No. 125 vol.13, FAO, Rome.

Imai, H., Cheng, J. H., Hamasaki, K., Numachi, K.-I. 2004. Identification of four mud crab species (genus *Scylla*) using ITS-1 and 16S rDNA markers. *Aquatic Living Resources*, **17**: 31-34.

Kaukinen, K. H., Supernault, K. J., Miller, K. M. 2004. Enrichment of tetranucleotide microsatellite loci from invertebrate species. *Journal of Shellfish Research*, **23**: 621-626.

Kawai, H. 1991. Current systems as the environment of aquatic animal. In: Kawai, H. ed., *Current Systems as the Environment of Aquatic Animals*. Kyoto University Press, Kyoto, pp.2-4. (in Japanese)

Lian, C., Wadud, M. A., Geng, Q., Shimatani, K., Hougetsu, T. 2006. An improved technique for isolating codominant compound microsatellite markers. *Journal of Plant Research*, **119**: 415-417.

Maggioni, R., Rogers, A. D. 2001. Microsatellite primers for three Western Atlantic *Farfantepenaeus* prawn species. *Molecular Ecology Notes*, **2**: 51-53.

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York, USA, p180.

Park, S. D. E. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. PhD thesis. University of Dublin, Dublin.

Pitcher, C. R. 1993. Spiny Lobster. In: Wright, A., Hill, L. (eds), *Nearshore Marine Resources of the South Pacific: Information for Fisheries Development and Management*. IPS (Suva)/ FFA(Honiara)/ ICOD (Canada). Chapter 17, pp 539-607.

Piry, S., Luikart, G., Cornuet, J. M. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**: 502-503.

Pritchard, J. K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**: 945-959.

Rozen, S., Skaletsky, H. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S., Misener, S. (eds), *Bioinformatics Methods and Protocols in the series Methods in Molecular Biology*. Humana Press, Totowa, NJ, pp.365-386.

Sekiguchi, H., Inoue, N. 2002. Recent advances in larval recruitment processes of scyllarid and palinurid lobsters in Japanese waters. *Journal of Oceanography*, **58**: 747-757.

Van Oosterhout, C. V., Hutchinson, W. F., Wills, D. P. M, Shipley P. 2004. MicroChecker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**: 535-538.

(Received June 26, 2017; Accepted September 3, 2017)