Genetic Diversity and Population Structure of Pronghorn Spiny Lobster *Panulirus penicillatus* in the Pacific Region[1]

Muhamad Fadry Abdullah,[2,7] *Seinen Chow*,[3] *Mitsuo Sakai*,[4] *Jin-Hua Cheng*,[5] *and Hideyuki Imai*[6]

Abstract: Adult specimens of *Panulirus penicillatus* were collected from localities distributed in Japan: Hachijojima Island, Ryukyu Archipelago (Amamioshima, Okinawajima, Ishigakijima); Taiwan: Taitung; Indonesia: Java Sea and Gebe Island; French Polynesia: Moorea Island; and Ecuador: Isabela Island. Phyllosoma larval specimens were collected from the western sea of the Galápagos Islands. In total, 569–570 nucleotide sequences were analyzed from the mtDNA control region of 480 individuals. Almost all individuals had a unique haplotype. Intrapopulation haplotype (h) and nucleotide (π) diversities were high for each locality, ranging $h = 0.9996–1.0000$ and $\pi = 0.0255–0.0448$. A mismatch distribution of pairwise differences between haplotypes indicated that *P. penicillatus* does not fit the sudden population expansion model. Analysis of molecular variance (AMOVA) shows a clear genetic difference between two regions (western/central Pacific and eastern Pacific). High gene flow was found within localities in the western/central Pacific region, probably resulting from an extended planktonic larval stage and prevailing ocean currents. In this study we detected genetic structure between eastern Pacific populations and western Pacific populations, indicating that despite the nearly 1-yr larval period for this species, larvae generally do not pass over the East Pacific Barrier.

The genus *Panulirus* White, 1874, has long been of interest to evolutionary biologists because of its high level of species diversity and its extensive geographic dispersal, as well as its importance as a fisheries commodity (Holthuis 1991, Ptacek et al. 2001, Abdullah et al. 2010). The species are found generally along continental coasts and around islands from tropical to temperate regions (George and Main 1967).

Among the spiny lobsters, the pronghorn spiny lobster, *Panulirus penicillatus* (Olivier, 1811), probably has the widest distribution. It is found in tropical and adjacent regions from southeastern Africa, the Red Sea, southern India, the Southeast Asian archipelago, Japan, northern Australia, and the southern and western Pacific islands, to Hawai'i, the Galápagos Islands, and other islands of the eastern Pacific (Holthuis 1991, Pitcher 1993). *Panulirus penicillatus* supports considerable fisheries in tropical Pacific regions. The total harvest of lobsters for inhabitants of the Pacific island nations is important, both economically and

[1] This study was supported in part by grants from the University of the Ryukyus Grants for International Research, the Sasagawa Scientific Research Grant from the Japan Science Society, and a grant for the Elucidation of "the Life History and Genetic Population Structure for Important Fisheries Fishes" from the Okinawa Prefectural Government. Manuscript accepted 1 July 2013.

[2] Graduate School of Engineering and Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan.

[3] National Research Institute of Fisheries Science, 2-12-4 Fukuura, Kanazawa, Yokohama, Kanagawa 236-8648, Japan.

[4] Tohoku National Fisheries Research Institute, 25-259 Shimomekurakubo, Hachinohe, Aomori 031-0841, Japan.

[5] Tungkang Biotechnology Research Center, Taiwan Fisheries Research Institute, Tungkang, Pingtung 92804, Taiwan.

[6] Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan.

[7] Corresponding author (e-mail: fadrynankeren@yahoo.com).

Pacific Science (2014), vol. 68, no. 2:197–211
doi:10.2984/68.2.3 (Includes online supplement)

socially, despite the relatively small amount of reported production in worldwide terms (Food and Agriculture Organization of the United Nations [FAO] 1987, Pitcher 1993). The recorded catch of the islands substantially underestimates the actual catch, primarily because a large proportion is consumed noncommercially. Furthermore, marketing systems are generally informal and diffuse (Pitcher 1993).

The identification of groups of interbreeding individuals as the basis for a fishery is a major issue in fisheries genetics (Thorpe et al. 2000). The genetic breeding unit is often known as a stock in fisheries biology (which is roughly the same as a population to a geneticist); this concept of stock has been discussed by many authors (e.g., Carvalho and Hauser 1995 and references therein; Thorpe et al. 2000). The common supposition is that within a stock there should be genetic homogeneity, and genetic divergence may occur between genetically isolated stocks over time (e.g., through drift, mutation, or selection) (Thorpe et al. 2000).

The assumptions that *P. penicillatus* phyllosoma larvae disperse widely and that unexploited stocks offer a protected supply for breeding stock recruitment should be evaluated before fisheries around most of the Pacific nations become fully developed. These apparently reasonable assumptions underlie existing knowledge about the robustness of the actual Pacific *P. penicillatus* stocks. The degree of certainty in these assumptions may differ across the region (Prescott 1988, Pitcher 1993) owing to the distance from and wide variety of adjacent reefs and the intensity of fishing in an area.

The early life history of spiny lobsters consists of a drifting larval period adapted for a relatively long-term stay in the open ocean, extending from several months to more than a year, with many possibilities for dispersal through ocean currents (Booth and Phillips 1994, Tolley et al. 2005). Much of the recent literature has focused on the role of the pelagic larval duration in determining realized dispersal distances and has resulted in mixed conclusions (Shanks et al. 2003, Bowen et al. 2006, Shanks 2009, Weersing and Toonen 2009, Riginos et al. 2011, Treml et al. 2012). For example, pelagic larval duration has been shown to be a strong predictor of dispersal distances (Shanks et al. 2003), but Weersing and Toonen (2009) suggested that general acceptance of pelagic larval duration as a reliable predictor of population connectivity across broad taxonomic lines is clearly unfounded. Indeed, pelagic larval duration was considerably correlated with dispersal distance, but there were many exceptions. Larval behavior can play a crucial role in determining dispersal distance (Shanks 2009), and type of eggs and life history parameters are also important predictors of connectivity in fishes (Bradbury et al. 2008, Riginos et al. 2011). Oceanographic currents are likely to create barriers and impact routes and directions of larval dispersal (Baums et al. 2006, Treml et al. 2008, Riginos et al. 2011).

The duration of the planktonic larval phase (phyllosoma) of *P. penicillatus* has been reported previously, based on field and laboratory studies. Using field-caught plankton samples, Johnson (1968) estimated the duration of the phyllosoma stage under natural conditions to be >7–8 months. Minagawa (1990) cultured phyllosoma of *P. penicillatus* under laboratory conditions and described the phases from hatching up to the midstages (reared for 160 days). The complete larval development of *P. penicillatus* in culture was subsequently documented by Matsuda et al. (2006), who reported a duration of 8.3–9.4 months for phyllosome larvae in culture.

Several molecular techniques, including allozymes, restriction fragment length polymorphism (RFLP), mitochondrial DNA sequencing, and microsatellites, have been used in studying genetic variability in marine organisms at the population level (Shaklee and Bentzen 1998, Diniz et al. 2005). Mitochondrial DNA sequencing, particularly of the most rapidly evolving and highly variable control region, has been a useful tool for population genetic studies of many terrestrial and aquatic organisms (Avise 1994). The control region, which includes the D-loop in vertebrates and is known as the AT-rich region in invertebrates, does not encode a functional protein and is therefore under fewer functional

and structural constraints, resulting in a high average substitution rate (Saccone et al. 1987, Diniz et al. 2005). Because it is generally the fastest-evolving region in the mitochondrial DNA of vertebrates and invertebrates, the control region is more sensitive than protein loci as a marker of phylogeographic structuring for many organisms (Avise 2000, Diniz et al. 2005).

Identification of phyllosoma of *P. penicillatus* from the western Pacific has been reported previously (Chow et al. 2006*a*,*b*). Further sequence analysis of cytochrome oxidase I and 16S rDNA from a Pacific population of *P. penicillatus* indicated two distinct subpopulations between the eastern and western/central Pacific; however, population analyses within the western/central Pacific and within the eastern Pacific were not possible because of the low variability in the markers and the small number of samples (Chow et al. 2011).

In the study reported here, we analyzed the highly polymorphic marker, mitochondrial DNA (mtDNA) control region of *P. penicillatus* to determine whether genetic structure could be detected in Pacific Ocean populations. Information regarding the contemporary population genetic structure combined with information on the life history of the species and the oceanographic history of the Pacific Ocean was used to infer larval distribution patterns and recent evolutionary history. Given the economic importance of *P. penicillatus*, this genetic information may be valuable for long-term fisheries management decisions.

MATERIALS AND METHODS

Lobster Samples

Adults of *Panulirus penicillatus* were collected from nine localities (see Table 1) throughout the western/central Pacific and eastern Pacific Ocean (Figure 1). Lobsters were purchased from local commercial fishers and fully complied with local fisheries management and marine protected area controls. Tissues samples from walking legs (pereiopods) or abdomen were dissected on site, immediately fixed in 70%–99% ethanol, and transferred to the laboratory. Phyllosoma larval samples of *P. penicillatus* from the western sea of the Galápagos Islands of the East Pacific Ocean were from the study by Chow et al. (2011).

DNA Analysis

About 50 mg of finely minced tissue sample was added to a 1.5 ml plastic test tube containing 0.5 ml TNES-8 M urea buffer (Asahida et al. 1996). After adding 10 μl proteinase K, it was incubated at 38ºC for 3 hr and then genomic DNA was extracted using phenol-chloroform procedure and precipitated with absolute ethanol according to the method described in Imai et al. (2004). The mtDNA control region was amplified using the polymerase chain reaction (PCR). Primers were designed manually specifically for *Panulirus penicillatus* (alignment of primer sequences against the Japanese spiny lobster *Panulirus japonicus*, DDBJ accession number AB071201). Several amplification attempts using the Panulirus12s primer (5′-TATAGCAAGAATCAAACTATAG), in the 12S ribosomal RNA gene, and the Panulirus-tRNA primer (5′-CATAGACGGGGTATGAGCCCGT), in the $tRNA^{met}$ gene, were unsuccessful. Thus, a new reverse primer, Penicillatus-R (5′-CATAGG(T/C)GTG(T/C)GAGGGAACAAAGTC), was based on the conservative nucleotide sequence of *P. penicillatus* obtained with primers Panulirus12s and Panulirus-tRNA. Primers Panulirus12s and Penicillatus-R were used for sequencing. PCR amplifications were performed in 50 μl reaction mixtures containing 1 μl template DNA, 12.5 pmol of each primer, 5 μl 10X Ex Taq reaction buffer, 5 μl dNTP mixture, and 2.5 unit Ex Taq Polymerase (Takara Bio Ltd.); the final volume of the reaction mixture was adjusted to 50 μl with sterile water. Reactions were performed in a thermal cycler (Perkin Elmer GeneAmp PCR System 9700) under conditions of an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 30 sec at 95°C, 30 sec at 50°C, and 1 min at 72°C, with a final 7-min extension at 72°C. PCR products were purified using a PCR Product Pre-sequencing kit (Exosap, USB Co.). The cleaned products were sequenced

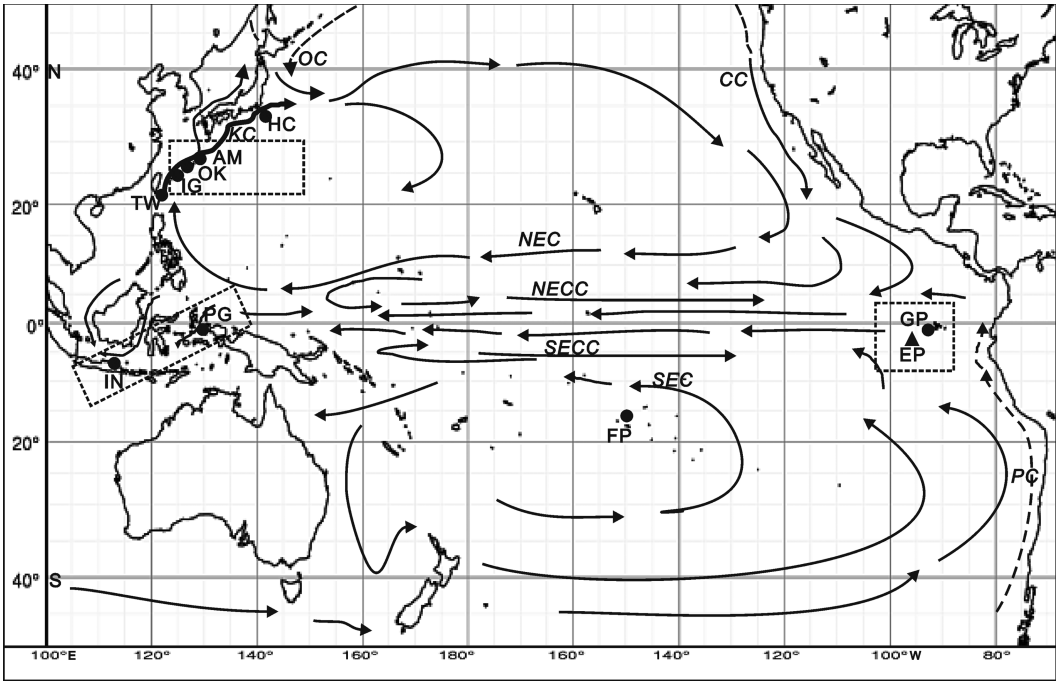

FIGURE 1. Map of the Pacific Ocean showing warm (solid lines) and cold currents (dashed lines), including the Oyashio Current (OC), Kuroshio Current (KC), North Equatorial Current (NEC), North Equatorial Counter Current (NECC), South Equatorial Counter Current (SECC), South Equatorial Current (SEC), California Current (CC), and Peru or Humboldt Current (PC). Dashed rectangles indicate population groupings (i.e., Ryukyu Archipelago, Indonesia, and East Pacific), closed circles indicate samples of adults, and the triangle indicates phyllosoma samples. Sampling site abbreviations correspond to those in Table 1. Ocean currents were drawn referring to Colling (2001).

on an ABI 3730xl Genetic Analyzer (Applied Biosystems) using a Big Dye Terminator Cycle Sequencing kit (ver. 3.1, Applied Biosystems).

Genetic Data and Analyses

The nucleotide sequence data reported in this paper appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB689204–AB689672. Sequence data were aligned using ClustalX (Thompson et al. 1997) with default alignment parameters and were checked manually for misalignments. The nucleotide compositions and numbers of variable sites were assessed with MEGA5 (Tamura et al. 2011). Haplotype and nucleotide diversity for each location were estimated using Arlequin (ver. 3.5) software (Excoffier et al. 2005). We ran preliminary analysis on each data set using analysis of molecular variance (AMOVA) in Arlequin (ver. 3.5) to measure standard diversity indices and pairwise F_{ST} (using haplotype frequency only) and Φ_{ST} (using genetic distance). Φ_{ST} was estimated using a Tamura-Nei (1993) distance method with a gamma correction ($\alpha = 0.27$) as determined by best nucleotide substitution models in MEGA5 (Tamura et al. 2011). The statistical significance of F_{ST} and Φ_{ST} value was tested by 1,000 permutations. In addition, several groupings were tested with SAMOVA (ver. 1.0) (Dupanloup et al. 2002), an annealing procedure to define clusters of adjacent populations that maximize the proportion of genetic variance due to differences between groups (F_{CT}), considering the geography of a region and oceanographic patterns. Due to Φ_{ST} being relatively unaffected by high allelic diversity, unlike F_{ST}

(Bird et al. 2011), we only report Φ_{ST} values. A neighbor-joining tree (Saitou and Nei 1987) based on Φ_{ST} values was constructed using Neighbor in Phylip (ver. 3.68) (Felsenstein 2004). The median-joining network (Bandelt et al. 1999) for the haplotypes was estimated using Network (ver 4.611) (http://www.fluxus-engineering.com).

Mismatch distribution differences were estimated to review historical demography. The shape of the mismatch distribution is relevant because it may be used to deduce whether a population is undergoing sudden population expansion or is in equilibrium (Rogers and Harpending 1992). Generally, chaotic and multimodal distributions suggest a population of constant size, whereas unimodal distributions reflect a population that has experienced a sudden demographic expansion. Parameters of the model of sudden expansion (τ, θ_0, θ_1) and mismatch distribution differences were estimated using Arlequin (ver. 3.5). Goodness-of-fit test was computed using Arlequin (ver. 3.5), calculated based on the sum of squared deviations (SSD) between observed and expected distribution under the sudden expansion, with the *P* value representing the probability of obtaining a simulated SSD equal to or larger than the observed one (Schneider and Excoffier 1999). Tajima's test of neutrality (Tajima 1989) was estimated using Arlequin (ver. 3.5), Tajima's *D* test is commonly used to test neutrality; however, it can also be used to examine population growth because population expansion may result in rejection of the null hypothesis of neutrality (significant negative *D* value).

RESULTS

Genetic Diversity

In total, sequence data of 569 base pairs (bp) (western/central Pacific region samples) and 570 bp (eastern Pacific region samples) were obtained from 480 specimens of *P. penicillatus*. Of the 571 aligned base pairs, 326 variable sites (excluding indels) were found: 246 were parsimony informative and 81 were singletons. In total, 469 haplotypes were identified, and nearly all sequenced individuals had a unique haplotype (Table 1). The nucleotide sequences of the haplotypes were deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers AB689204–AB689672.

The average nucleotide composition of the control region was 38.1% adenine, 31.6% thymine, 19.3% cytosine, and 11% guanine, consistent with previous reports of an AT-rich control region of the mitochondrial genome in many invertebrates, including crustaceans (McMillen-Jackson and Bert 2004, Diniz et al. 2005). Haplotype diversity (h) within the geographical populations was high, ranging from 0.9996 in the Ryukyu Archipelago to 1.0000 in Hachijojima Island, Taiwan, and French Polynesia. Nucleotide diversity (π) was generally high, ranging from 0.0300 in the East Pacific to 0.0582 in Hachijojima Island (Table 1). Four of the shared haplotypes were represented at two geographical sites, and the other two were shared only between individuals restricted to the same geographical site (Supplemental Appendix S1).

Authors' Note: Supplemental Appendix S1 available only on BioOne (http://www.bioone.org/).

Structure of the Pronghorn Spiny Lobster Population

Individuals were binned into six groups of populations defined by geographical region and oceanographic history (i.e., exposure to currents): Hachijojima Island ($n = 45$); Amamioshima, Okinawajima, and Ishigakijima Islands in the Ryukyu Archipelago (162); Taiwan (47); Java Sea and Gebe Island in Indonesia (97); French Polynesia (47); and Isabela Island and phyllosome larvae from the western sea of the Galápagos Islands in the East Pacific (81). In addition, SAMOVA maximized at two groups, with the Isabela Island and phyllosome larvae from the western sea of the Galápagos Islands in the East Pacific grouped separately from the rest of the sampling sites ($F_{CT} = 0.76$, $P < .05$). An AMOVA based on conventional *F* statistics (haplotype frequency only) failed to detect any population structure, but an AMOVA using the Tamura-Nei (1993) distance performed on the mtDNA sequence data set revealed a

TABLE 1

Sample Abbreviation (ID), Sample Locality, Sample Size (*N*), Year of Collection, Intrapopulation Haplotype Diversity (h), and Nucleotide Diversity (π) for *Panulirus penicillatus* Mitochondrial DNA Control Region

Sample ID	Locality	*N*	Year	Population	No. of Haplotypes	h	π	Population Region
HC	Hachijojima Islands, Japan	45	2009	Hachijojima Island	45	1.0000 ± 0.0047	0.0582 ± 0.0287	Western/central Pacific
AM	Amamioshima Islands, Japan	50	2008	Ryukyu Archipelago	157	0.9996 ± 0.0007	0.0581 ± 0.0282	
OK	Okinawajima Islands, Japan	59	2008					
IG	Ishigakijima Islands, Japan	53	2008					
TW	Taiwan	47	2009	Taiwan	47	1.0000 ± 0.0044	0.0525 ± 0.0259	
IN	Java Sea, Indonesia	55	2008	Indonesia	97	0.9998 ± 0.0015	0.0536 ± 0.0261	
PG	Gebe Island, Indonesia	43	2010					
FP	Moorea, French Polynesia	47	2011	French Polynesia	47	1.0000 ± 0.0044	0.0498 ± 0.0246	
GP	Isabela Island, Ecuador	48	2010	East Pacific	80	0.9997 ± 0.0020	0.0300 ± 0.0150	Eastern Pacific
EP[a]	Western sea of the Galápagos Islands, Ecuador	33	2008					
Total		480						

Note: Populations were binned from sampling locality to six groups of population defined by geographical region and oceanographic history. See text for details.

[a] Phyllosoma larval samples were from the study by Chow et al. (2011).

TABLE 2

Pairwise Φ_{ST} Values (Above the Diagonal) and Pairwise Φ_{ST} *P* Values (Below the Diagonal) of Mitochondrial DNA Control Region among Populations of *Panulirus penicillatus*

Locality	Hachijojima Island	Ryukyu Archipelago	Taiwan	Indonesia	French Polynesia	East Pacific
Hachijojima Island		0.00159	0.01011	0.00094	0.00944	0.88118
Ryukyu Archipelago	.21622		–0.00430	–0.00005	–0.00338	0.85811
Taiwan	.1081	.85586		–0.00121	–0.00966	0.88506
Indonesia	.36036	.34234	.59459		0.00075	0.87229
French Polynesia	.16216	.78378	.95495	.34234		0.88886
East Pacific	.00000*	.00000*	.00000*	.00000*	.00000*	

* $P < .05$.

structure in population. There was no significant population distinction within samples from western/central Pacific populations by AMOVA. The pairwise Φ_{ST} among populations were high between eastern Pacific and western/central Pacific populations (0.858–0.889) (Table 2). Congruent with the pattern observed in the network haplotypes (Plate I), we found strong regional structuring between eastern and western/central Pacific populations of *P. penicillatus*, which explained most genetic variations (85%) between the regions. To analyze the substructure in the western/central Pacific area we attempted three types of regional grouping: (1) Hachijojima-Ryukyu-Taiwan-Indonesia and French Polynesia, (2) Hachijojima-Ryukyu-Taiwan and Indonesia–French Polynesia, and (3) Hachijojima-Ryukyu-Taiwan, Indonesia, and French Polynesia. Regarding these groupings, genetic variation and Φ_{ST} among populations was small (% var. –0.19 to –0.11) and did not differ significantly from 0 (Table 3). A neighbor-joining tree based on Φ_{ST} values showed a prominent genetic break between western/central Pacific and eastern Pacific populations (Figure 2). Based on the goodness-of-fit test, *P. penicillatus* could be fitted to an expansion model (all the population has $P > .05$) (Figure 3). However, this method is quite conservative, rarely rejecting the expansion model (Schneider and Excoffier 1999). Indeed, visual inspection of the mismatch distributions suggested a multimodal profile, as is typical of demographically stable populations (Rogers and Harpending 1992). The mismatch distribution for the entire sample deviated significantly from the expected distribution under a sudden expansion model. This outcome is supported by a lack of significance with Tajima's *D* test, except for eastern Pacific samples, where Tajima's test showed significant negative values ($D = -1.557$, $P = .039$). Appropriate dating of population expansions was not possible because mutation rates for the mtDNA control region in this species have not been estimated. Thus, the mutation rate was calculated according to Babbucci et al. (2010), ranging between 8.1% and 13.4% per million years, and applied to the model $T = \tau/2u$ (Rogers and Harpending 1992), where T = time since expansion, $2u = \mu \times$ number bases sequenced × generation time (3 yr generation). A rough estimation suggested that population expansions occurred in the late Pleistocene: 52,000 to 200,000 yr ago for western/central Pacific *P. penicillatus* and 41,000 to 68,000 yr ago for the eastern Pacific.

DISCUSSION

Mitochondrial DNA (mtDNA) has been widely used for studies of genetic differentiation in the spiny lobster genus *Panulirus*. Genetic differences were not detected between samples of *P. japonicus* along the Japanese coast, supporting the hypothesis that benthic individuals of *P. japonicus* are sustained from a common pool of long-period phyllosoma

TABLE 3

AMOVA Results Showing Degrees of Freedom (df), Variance Components (Var.), Percentage Variation (% Var.) and Φ Statistics for *Panulirus penicillatus*

Locality	df	Var.	% Var.	Φ Statistics	*P* Value
All sites (western-central/eastern Pacific)					
Among regions	1	84.19733	85.23	$\Phi_{CT} = 0.85226$	.16129
Among populations	4	0.00997	0.01	$\Phi_{SC} = 0.00068$	.51222
Within populations	474	14.58564	14.76	$\Phi_{ST} = 0.85236$	.00000
Western-central Pacific region					
(Hachijojima-Ryukyu-Taiwan-Indonesia/French	1	–0.0303	–0.19	$\Phi_{CT} = -0.00193$	.78495
Polynesia)	3	0.00192	0.01	$\Phi_{SC} = 0.00012$	.43304
Among regions	394	15.75562	100.18	$\Phi_{ST} = -0.00181$	.49853
Among populations					
Within populations					
(Hachijojima-Ryukyu-Taiwan/Indonesia-French Polynesia)					
Among regions	1	–0.01748	–0.11	$\Phi_{CT} = -0.00111$	.70283
Among populations	3	0.00435	0.03	$\Phi_{SC} = 0.00028$	.44673
Within populations	394	15.75562	100.08	$\Phi_{ST} = -0.00083$	.50538
(Hachijojima-Ryukyu-Taiwan/Indonesia/French Polynesia)					
Among regions	2	–0.02017	–0.13	$\Phi_{CT} = -0.00128$	.47019
Among populations	2	0.00763	0.05	$\Phi_{SC} = -0.00080$	.44477
Within populations	394	15.75562	100.08	$\Phi_{ST} = -0.00080$	.52395

Note: Runs included all populations, as described in the text (1,000 permutations).

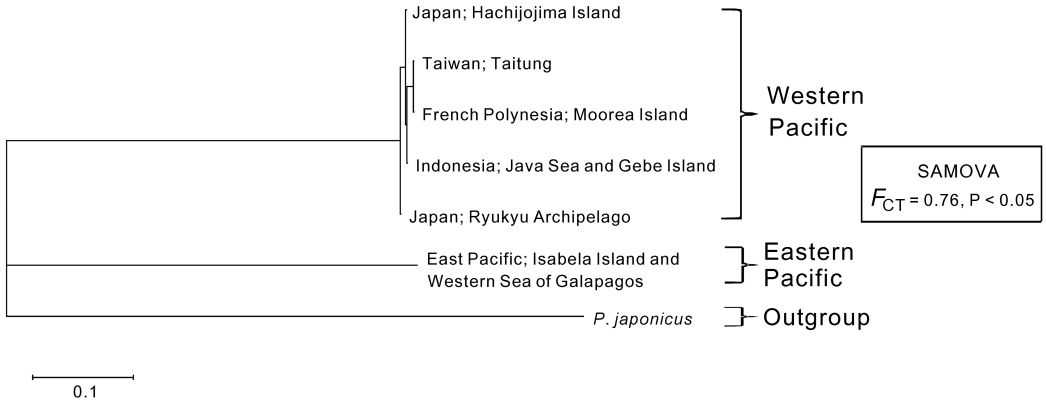

FIGURE 2. Neighbor-joining population tree based on Φ_{ST} values, illustrating the most probable geographical structure in the analysis of molecular variance (AMOVA). The proportion of genetic variance due to differences between groups (F_{CT}) of the western/central and eastern Pacific was calculated using SAMOVA. The results suggest that there is high gene flow between populations of *Panulirus penicillatus* in the western/central Pacific region, whereas restricted gene flow was found between populations of the western/central Pacific region and eastern Pacific region. The Japanese spiny lobster *P. japonicus* was used as outgroup.

larvae through long-distance larval transport within the Kuroshio Subgyre (Inoue et al. 2007). High genetic variability of the blue spiny lobster, *P. inflatus*, along the Pacific coast of Mexico has been observed. Furthermore, it has been suggested that the lack of genetic population structure is related to oceanographic flows in the area, coupled with a long larval period (García-Rodríguez and Perez-Enriquez 2008). A high level of diver-

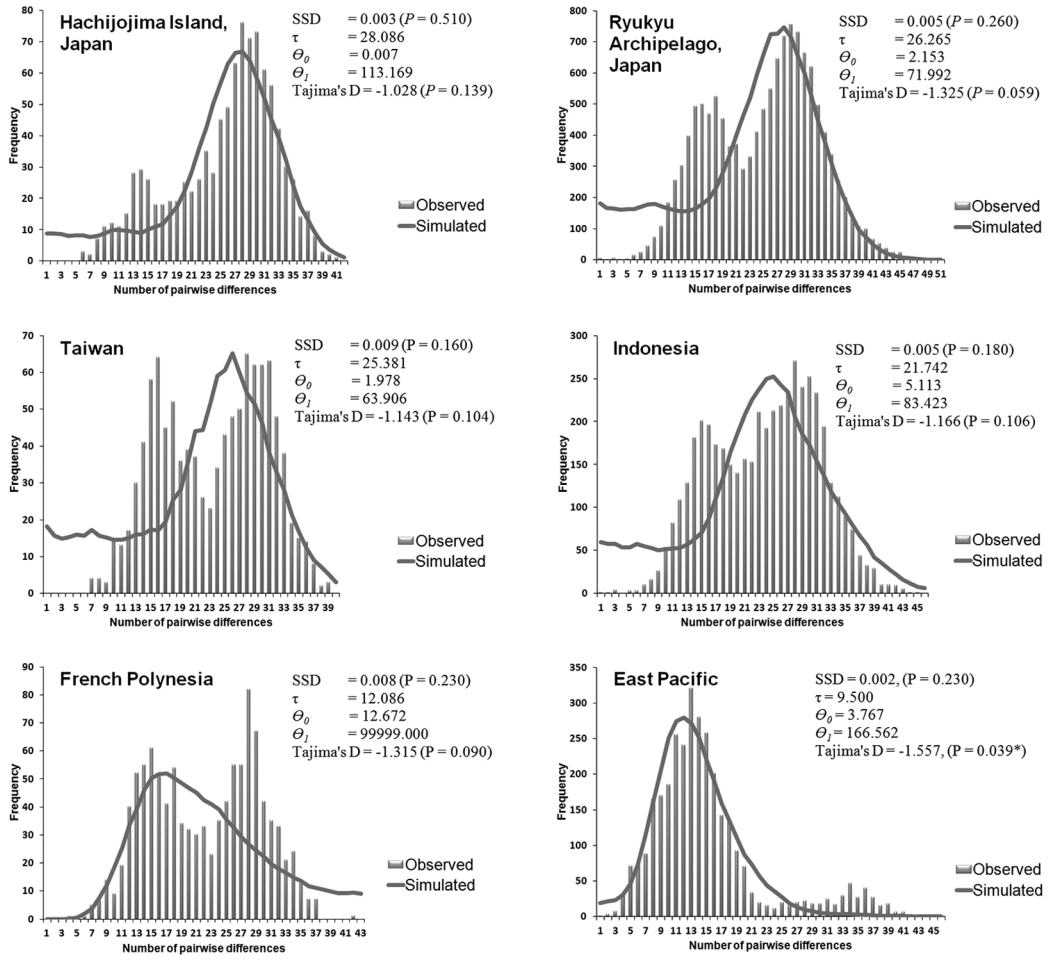

FIGURE 3. Mismatch distribution established for *Panulirus penicillatus* mitochondrial control region. Bars represent the observed frequency of the pairwise differences among haplotypes, and the line shows the expected curve predicted for a population that has undergone a demographic expansion in the past. The parameters of the model of sudden expansion (Rogers and Harpending 1992) are presented as well as goodness-of- fit test to the model: SSD sum of squared deviations; θ_0, pre-expansion and θ_1, postexpansion population sizes; τ, time in number of generations elapsed since the sudden expansion episode. Tajima's (1989) *D* test value and its statistical significance are also given.

gence was also observed between Brazilian and northwestern Atlantic populations of *P. argus*, and this genetic distinction may have arisen due to the pattern of the South Equatorial Current in the area (Sarver et al. 1998, Diniz et al. 2005).

Water movement patterns have also been suggested to influence the genetic population structures of other crustaceans. In the red rock lobster, *Jasus edwardsii*, which occurs in southern Australia and New Zealand, mtDNA assessment failed to detect genetic subdivisions of populations spanning 4,500 km of its range (Ovenden et al. 1992). The currents leaving southern Australia and flowing past New Zealand may transport larvae masses. However, in the green rock lobster, *Sagmariasus verreauxi* (*J. verreauxi* synonym), genetic distinctions were detected between Australian and New Zealand populations using mtDNA

(Brasher et al. 1992, Chan 2010). Previous study of *P. penicillatus* using analysis of COI and 16s rDNA found two distinct subpopulations between the eastern and western/central Pacific (Chow et al. 2011). However, population analyses within the western/central Pacific and within the eastern Pacific were not possible due to low variability of markers and small number of samples.

In the study reported here, the mtDNA control region exhibited high genetic variability ($h = 0.9996$ to 1.0000 and $\pi = 0.0300$ to 0.0581); these are relatively high values compared with the control region diversities of other *Panulirus* spp. (*P. inflatus:* $h = 0.957$ to 1.0000 and $\pi = 0.019$ to 0.028 [García-Rodríguez and Perez-Enriquez 2008]; *P. argus:* $h = 0.667$ to 1.0000 and $\pi = 0.003$ to 0.066 [Diniz et al. 2005]). The control region is a noncoding region that includes signals necessary for replication of a molecule and is the most rapidly evolving region of mtDNA (Heyer et al. 2001). The high genetic variability of the control region in *P. penicillatus* may be maintained by its large population size. In fact, because nearly all individuals represented a unique haplotype, an investigation based on sequencing the control region would require a very large sample size to detect common haplotypes and associate them with particular geographic areas (García-Rodríguez and Perez-Enriquez 2008). The evolutionary pattern of interhaplotypic divergence will almost always produce a biogeographic signature, and an analysis that recognizes genetic distance among alleles will provide additional resolution, especially for hypervariable markers (Bird et al. 2011).

Significant genetic divergence was found between eastern and western/central Pacific populations based on polymorphic markers (the control region). Our statistical assignment of genetic heterogeneity among populations found genetic isolation only between *P. penicillatus* from the eastern Pacific and western/central Pacific, with no genetic structuring observed within each region (Table 3). For *P. penicillatus* differentiation of the eastern Pacific and western/central Pacific with respect to mtDNA traits may be due to contemporary isolation/gene flow or prior isolation with recent gene flow, although the overall level of genetic exchange must be below that required to homogenize the populations.

No population structure was found within the population of *P. penicillatus* inhabiting the western Pacific to the central Pacific: Hachijojima Island, Ryukyu Archipelago, Taiwan, Indonesia, and French Polynesia. Genetic similarities were also shown in this region for other marine organisms such as coral, fish, and starfish (*Acanthaster planci*) (Rodriguez-Lanetty and Hoegh-Guldberg 2002, Mukai et al. 2009, Yasuda et al. 2009). Furthermore, another *Panulirus* species, *P. japonicus*, was found to be sustained from a common pool of long-period phyllosoma larvae through long-distance larval transport within the Kuroshio Subgyre (Inoue et al. 2007). For *P. penicillatus*, long larval life (nearly 1 yr) of the phyllosoma (Johnson 1968, Booth and Phillips 1994, Matsuda 2006) coupled with the considerable impact of oceanic current systems or gyres (e.g., the Kuroshio and Equatorial Currents) may explain the observed patterns. The Kuroshio Current, a strong western boundary current, flows northeastward and is suspected to transport larvae to the northwestern Pacific. Furthermore, transportation of larvae between the western-central Pacific more likely occurred indirectly via stepping-stones by the Equatorial Currents, and small amounts of larval dispersal over years might result in genetic homogeneity. Unfortunately, population analysis within the eastern Pacific could not be done because there were only two locations in this study.

Predicting connectivity of marine populations is extremely difficult due to the unknown and variable biology of larvae, the complex physical environment, and the inherent difficulties in considering the range in spatial and temporal scales covered by larval dispersal (Werner et al. 2007, Botsford et al. 2009, Treml et al. 2012). Currently, biophysical modeling approaches have been used to understand the spatial patterns in connectivity by integrating seascape data with a species' life-history characteristics (Treml et al. 2008, White et al. 2010, Kool et al. 2011). Biophysical studies demonstrate how complex oceanographic information can be used to

provide insight into the evolution of genetic structure in marine environments (Kool et al. 2011).

Panulirus penicillatus has been reported to be commercially abundant in the eastern Pacific, particularly in the Galápagos Islands (George and Main 1967, Holthuis and Loesch 1967). *Panulirus penicillatus* represents more than 75% of the annual spiny lobster catch in the Galápagos Islands, which in recent years has shown indications of decline in abundance (Hearn and Murillo 2008). The phyllosoma larvae of *P. penicillatus* have been found throughout the tropical eastern Pacific some 3,500–4,000 km west of the nearest likely source (the Galápagos Islands); this suggests the apparent crossing of the East Pacific Barrier, a vast stretch of open ocean that divides the majority of Pacific islands from those to the east and corresponds to a major break in faunal composition (Ekman 1953, Johnson 1974, Pitcher 1993). Johnson (1974) also indicated that the countercurrents within the Equatorial Current System (Figure 1) could provide possible routes for the return of eastern Pacific species that have either fortuitously or instinctively shifted into countercurrents, possibly through habits of diurnal migrations, and that have not drifted westward to a point of no return, determined by requirements inherent in their life cycle. In the study reported here we detected genetic structure between eastern Pacific populations and western/central Pacific populations, indicating that despite the nearly 1-yr larval period for this species (e.g., Booth and Phillips 1994, George 2005), the larvae generally could not pass over the East Pacific Barrier, or they could regularly cross this barrier but failed to survive in appreciable numbers in other regions due to some selective advantage of local recruits and habitat preferences. The absence of intermediate "stepping-stones" (areas of available adult habitat that provide generational layovers between the end of one dispersal event and the beginning of another population) can represent a major barrier to dispersal and gene flow (Crandall et al. 2012). More important, the lack of shared mtDNA haplotypes and large Φ_{ST} values suggest that there has been prolonged historical isolation between the western/central Pacific and eastern Pacific populations.

Further analysis on recruitment of *P. penicillatus* phyllosoma larvae may be necessary because genetic distinction in eastern Pacific populations may indicate the potential self-recruitment of phyllosoma larvae in this region. Although phyllosoma larvae are weak swimmers, they have the ability to maintain their vertical position in the water column (Johnson 1974). Larval dispersal for spiny lobsters has been previously modeled. Griffin et al. (2001) discovered that *P. cygnus* was a self-recruiting species, restricted to Western Australia. A more recent simulation study of *P. argus* found the average dispersal of *P. argus* in the Caribbean Sea to be possibly only 200–400 km, suggesting that larval behavior such as vertical migration, together with a retentive oceanographic environment, may increase the potential for self-recruitment (Butler et al. 2011).

Adult *P. penicillatus* show variations in body color, including yellow green, brown green, blue black, and dark reddish brown (Holthuis 1991, George 2006). The dark reddish-brown form is regionally limited to the eastern Pacific, within the Galápagos, Clipperton, Socorro, and Revillagigedo Islands, where it is known as the "red lobster" (Holthuis and Loesch 1967, Holthuis 1991, George 2006). It is probably restricted to the eastern Pacific because of equatorial currents. Consistent with previous findings, we identified morphological variability in the body color of *P. penicillatus* species: eastern Pacific populations had a reddish body and western/central Pacific populations had a greenish body (Plate I). George (2006) proposed that the genetic distinctions for subpopulations of *P. penicillatus* from the eastern Pacific, referred to as *P. penicillatus* red, could be explained by assuming that the East Pacific Barrier of Ekman (1953) represents a major barrier that maintains the isolation of these subpopulations. However, further research is recommended to clearly establish the correlation in the variation of morphological color in this species.

Historical demographic parameters of *P. penicillatus* from western/central Pacific populations reveal a more stable population history

compared with populations from the eastern Pacific. The expansion of the *P. penicillatus* population was estimated to have occurred 52,000 to 200,000 yr ago for the western/central Pacific and 41,000 to 68,000 yr ago for the eastern Pacific during the Pleistocene. The late Pleistocene Period (the past 1 million yr) was punctuated by a series of large glacial and interglacial changes (Imbrie et al. 1992).

The pattern of mismatched distributions of *P. penicillatus* differed between the western/central Pacific and eastern Pacific regions, and the expansion age parameter (τ) was lower in the eastern Pacific region. Those tendencies may results from higher diversity and size of the population in the western/central Pacific, which could maintain diversity as a metapopulation, whereas the eastern Pacific population is smaller and probably more recent in origin.

ACKNOWLEDGMENTS

We are grateful to Serge Planes, David Lecchini, Héloïse Rouzé, Teva Lechat, and colleagues (Centre de Recherches Insulaires et Observatoire de l'Environnement de Polynésie Française, CRIOBE, Moorea, French Polynesia) for their invaluable support during spiny lobster sampling in French Polynesia. We are also grateful to Hiroji Ueki (Electromarina Cia. Ltda., Equador), Isoyabussan Ltd., and Radi Ihlas Albani (Seaweed Culture Program, Gebe, Indonesia) for collecting spiny lobsters at Isabela Island, Ecuador, Java Sea, and Gebe Island, Indonesia, respectively. We thank Hiroshi Inoue and Kensuke Iwamoto (University of the Ryukyus, Japan) for help on lobster sampling at Okinawajima Island. We are also grateful to Hideo Sekiguchi (Mie University, Japan) for providing a reference.

Literature Cited

Abdullah, M. F., H. Inoue, K. Iwamoto, and H. Imai. 2010. A new record of *Panulirus longipes longipes* (A. Milne-Edwards, 1868) (Decapoda: Palinuridae) at Okinawajima Island and the phylogenetic position of the genus *Panulirus* using RAPD markers. Biol. Mag. Okinawa 48:77–89.

Asahida, T., T. Kobayashi, K. Saitoh, and I. Nakayama. 1996. Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. Fish. Sci. 62:727–730.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, London.

———. 2000. Phylogeography: The history and formation of species. Harvard University Press, Cambridge, Massachusetts.

Babbucci, M., S. Buccoli, A. Cau, R. Cannas, R. Goñi, D. Díaz, S. Marcato, L. Zane, and T. Patarnello. 2010. Population structure, demographic history, and selective processes: Contrasting evidences from mitochondrial and nuclear markers in the European spiny lobster *Palinurus elephas* (Fabricius, 1787). Mol. Phylogenet. Evol. 56 (3): 1040–1050.

Bandelt, H. J., P. Forster, and A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16:37–48.

Baums, I. B., C. B. Paris, and L. M. Cherubin. 2006. A biooceanographic filter to larval dispersal in a reef-building coral. Limnol. Oceanogr. 51:1969–1981.

Bird, C. E., P. E. Smouse, S. A. Karl, and R. J. Toonen. 2011. Detecting and measuring genetic differentiation. Pages 31–55 *in* S. Koenemann, C. Held, and C. Schubart, eds. Crustacean issues: Phylogeography and population genetics in Crustacea. CRC Press, Boca Raton, Florida.

Booth, J. D., and B. F. Phillips. 1994. Early life history of spiny lobster. Crustaceana (Leiden) 66:271–294.

Botsford, L. W., D. R. Brumbaugh, C. Grimes, J. B. Kellner, J. Largier, M. R. O'Farrell, S. Ralston, E. Soulanille, and V. Wespestad. 2009. Connectivity, sustainability, and yield: Bridging the gap between conventional fisheries management and marine protected areas. Rev. Fish Biol. Fish. 19:69–95.

Bowen, B. W., A. L. Bass, A. Muss, J. Carlin, and D. R. Robertson. 2006. Phylogeogra-

phy of two Atlantic squirrelfishes (Family Holocentridae): Exploring links between pelagic larval duration and population connectivity. Mar. Biol. (Berl.) 149:899–913.

Bradbury, I. R., B. Laurel, P. V. R. Snelgrove, P. Bentzen, and S. E. Campana. 2008. Global patterns in marine dispersal estimates: The influence of geography, taxonomic category and life history. Proc. R. Soc. Lond. B Biol. Sci. 275:1803–1809.

Brasher, D. J., J. R. Ovenden, J. D. Booth, and R. W. G. White. 1992. Genetic subdivision of Australian and New Zealand population of *Jasus verreauxi* (Decapoda; Palinuridae): Preliminary evidence from the mitochondrial genome. N. Z. J. Mar. Freshwater Res. 26:53–58.

Butler IV, M. J., C. B. Paris, J. S. Goldstein, H. Matsuda, and R. K. Cowen. 2011. Behavior constrains the dispersal of long-lived spiny lobster larvae. Mar. Ecol. Prog. Ser. 422:223–237.

Carvalho, G. R., and L. Hauser. 1995. Molecular genetics and the stock concept in fisheries. Pages 55–79 *in* G. R. Carvalho and T. J. Pitcher, eds. Molecular genetics in fisheries. Chapman and Hall, London.

Chan, T. Y. 2010. Annotated checklist of the world's marine lobsters (Crustacea: Decapoda: Astacidea, Glypheidea, Achelata, Polychelida). Raff. Bull. Zool. Suppl. 23:153–181.

Chow, S., A. Jeffs, Y. Miyake, K. Konishi, M. Okazaki, N. Suzuki, S. Kimura, M. F. Abdullah, H. Imai, M. Sasaki, Y. Sasai, T. Wakabayasi, and M. Sakai. 2011. Genetic isolation between the western and eastern Pacific populations of pronghorn spiny lobster *Panulirus penicillatus*. PLoS ONE 6(12): e29280. doi:10.1371/journal.pone.0029280.

Chow, S., N. Suzuki, H. Imai, and T. Yoshimura. 2006*a*. Molecular species identification of spiny lobster phyllosoma larvae of the genus *Panulirus* from the northwestern Pacific. Mar. Biotechnol. 8:260–267.

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

Colling, A. 2001. Ocean circulation. 2nd ed. The Open University and Butterworth-Heinemann, Milton-Keynes.

Crandall, E. D., E. A. Treml, and P. H. Barber. 2012. Coalescent and biophysical models of stepping-stone gene flow in neritid snails. Mol. Ecol. 21:5579–5598.

Diniz, F. M., N. Maclean, M. Ogawa, I. H. A. Cintra, and P. Bentzen. 2005. The hypervariable domain of the mitochondrial control region in Atlantic spiny lobsters and its potential as a marker for investigating phylogeographic structuring. Mar. Biotechnol. 7:462–473.

Dupanloup, I., S. Schneider, and L. Excoffier. 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11:2571–2581.

Ekman, S. 1953. Zoogeography of the sea. Sidgwick and Jackson, London.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online 1:47–50.

Food and Agriculture Organization of the United Nations (FAO). 1989. FAO Yearbook in 1987 of fishery statistics, catches and landings. FAO Yearbook, Vol. 64.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

García-Rodríguez, F. J., and R. Perez-Enriquez. 2008. Lack of genetic differentiation of blue spiny lobster *Panulirus inflatus* along the Pacific coast of Mexico inferred from the mtDNA sequences. Mar. Ecol. Prog. Ser. 361:203–212.

George, R. W. 2005. Evolution of life cycles, including migration, in spiny lobsters (Palinuridae). N. Z. J. Mar. Freshwater Res. 39:503–514.

———. 2006. Tethys sea fragmentation and speciation of *Panulirus* spiny lobsters. Crustaceana (Leiden) 78:1281–1309.

George, R. W., and A. R. Main. 1967. The evolution of spiny lobsters (Palinuridae): A study of evolution in the marine environment. Evolution 21:803–820.

Griffin, D. A., J. L. Wilkin, C. F. Chubb, A. F. Pearce, and N. Caputi. 2001. Ocean

currents and the larval phase of Australian western rock lobster, *Panulirus cygnus*. Mar. Freshwater Res. 52:1187–1199.

Hearn, A., and J. C. Murillo. 2008. Life history of the red spiny lobster, *Panulirus penicillatus* (Decapoda: Palinuridae), in the Galápagos Marine Reserve, Ecuador. Pac. Sci. 62:191–204.

Heyer, E., E. Zietkiewicz, A. Rochowski, V. Yotova, J. Puymirat, and D. Labuda. 2001. Phylogenetic and familial estimates of mitochondrial substitution rates: Study of control region mutations in deep-rooting pedigrees. Am. J. Hum. Genet. 69:1113–1126.

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. Synop. No. 125, Vol. 13.

Holthuis, L. B., and H. Loesch. 1967. Lobsters of the Galápagos Islands (Decapoda, Palinuridea). Crustaceana (Leiden) 12:214–222.

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

Imbrie, J., E. A. Boyle, S. C. Clemens, A. Duffy, W. R. Howard, G. Kukla, J. Kutzbach, D. G. Martinson, A. McIntyre, A. C. Mix, B. Molfino, J. J. Morley, L. C. Peterson, N. G. Pisias, W. L. Prell, M. E. Raymo, N. J. Shackleton, and J. R. Toggweiler. 1992. On the structure and origin of major glaciation cycles, 1. Linear responses to Milankovitch forcing. Paleoceanography 7:701–738.

Inoue, N., H. Watanabe, S. Kojima, and H. Sekiguchi. 2007. Population structure of Japanese spiny lobster *Panulirus japonicus* inferred by nucleotide sequence analysis of mitochondrial COI gene. Fish. Sci. 73:550–556.

Johnson, M. W. 1968. Palinurid phyllosoma from the Hawaiian archipelago (Palinuridae). Crustaceana Suppl. (Leiden) 2:59–79.

———. 1974. On the dispersal of lobster larvae into the East Pacific Barrier (Decapoda, Palinuridea). Fish. Bull. 72:639–647.

Kool, J. T., C. B. Paris, P. H. Barber, and R. K. Cowen. 2011. Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. Glob. Ecol. Biogeogr. 20:695–706.

Matsuda, H., T. Takenouchi, and J. S. Goldstein. 2006. The complete larval development of the pronghorn spiny lobster *Panulirus penicillatus* (Decapoda: Palinuridae) in culture. J. Crustacean Biol. 26:579–600.

McMillen-Jackson, A. L., and T. M. Bert. 2004. Genetic diversity in the mtDNA control region and population structure in the pink shrimp *Farfantepenaeus duorarum*. J. Crustacean Biol. 24:101–109.

Minagawa, M. 1990. Early and middle larval development of *Panulirus penicillatus* (Olivier) (Crustacea, Decapoda, Palinuridae) reared in the laboratory. Res. Crustacea 18:77–93.

Mukai, T., S. Nakamura, and M. Nishida. 2009. Genetic population structure of a reef goby, *Bathygobius cocosensis*, in the northwestern Pacific. Ichthyol. Res. 56:380–387.

Ovenden, J. R., D. J. Brasher, and R. W. G. White. 1992. Mitochondrial DNA analyses of the red rock lobster *Jasus edwardsii* supports an apparent absence of population subdivision throughout Australasia. Mar. Biol. (Berl.) 112:319–326.

Pitcher, C. R. 1993. Spiny lobster. Pages 539–607 *in* A. Wright and L. Hill, eds. Nearshore marine resources of the South Pacific: Information for fisheries development and management. IPS (Suva)/FFA (Honiara)/ICOD (Canada).

Prescott, J. 1988. Tropical spiny lobster: An overview of their biology, the fisheries and the economics with particular reference to the double spined rock lobster *P. penicillatus*. South Pacific Commission Workshop. Pacific Inshore Fishery Resources, New Caledonia. Working Paper 18:1–36.

Ptacek, M. B., S. K. Sarver, M. J. Childress, and W. F. Herrnkind. 2001. Molecular phylogeny of the spiny lobster genus *Panulirus* (Decapoda: Palinuridae). Mar. Freshwater Res. 52:1037–1047.

Riginos, C., K. E. Douglas, Y. Jin, D. F. Shanahan, and E. A. Treml. 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography 34:566–575.

Rodriguez-Lanetty, M., and O. Hoegh-Guldberg. 2002. The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Plesiastrea versipora* in the western Pacific. Mol. Ecol. 11:1177–1189.

Rogers, A. R., and H. Harpending. 1992. Population growth waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552–569.

Saccone, C., M. Attimonelli, and E. Sbisa. 1987. Structural elements highly preserved during the evolution of the D-loop-containing region in vertebrate mitochondrial DNA. J. Mol. Evol. 26:205–211.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

Sarver, S., J. D. Silberman, and P. J. Walsh. 1998. Mitochondrial DNA sequence evidence supporting the recognition of two subspecies or species of the Florida spiny lobster *Panulirus argus*. J. Crustacean Biol. 18:177–186.

Schneider, S., and L. Excoffier. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA. Genetics 152:1079–1089.

Shaklee, J. B., and P. Bentzen. 1998. Genetic identification of stocks of marine fish and shellfish. Bull. Mar. Sci. 62:589–621.

Shanks, A. L. 2009. Pelagic larval duration and dispersal distance revisited. Biol. Bull. (Woods Hole) 216:373–385.

Shanks, A. L., B. A. Grantham, and M. H. Carr. 2003. Propagule dispersal distance and the size and spacing of marine reserves. Ecol. Appl. 13:S159–169.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731–2739.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876–4882.

Thorpe, J. P., A. M. Solé-Cava, and P. C. Watts. 2000. Exploited marine invertebrates: Genetics and fisheries. Hydrobiologia 420:165–184.

Tolley, K. A., J. C. Groeneveld, K. Gopal, and C. A. Matthee. 2005. Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. Mar. Ecol. Prog. Ser. 297:225–231.

Treml, E. A., P. N. Halpin, D. L. Urban, and L. F. Pratson. 2008. Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landscape Ecol. 23:19–36.

Treml, E. A., J. J. Roberts, Y. Chao, P. N. Halpin, H. P. Possingham, and C. Riginos. 2012. Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations. Integr. Comp. Biol. 52:525–537.

Weersing, K., and R. J. Toonen. 2009. Population genetics, larval dispersal, and connectivity in marine systems. Mar. Ecol. Prog. Ser. 393:1–12.

Werner, F. E., R. K. Cowen, and C. B. Paris. 2007. Coupled biological and physical models: Present capabilities and necessary developments for future studies of population connectivity. Oceanography Spec. Issue: Marine Population Connectivity 20:54–69.

White, C., K. A. Selkoe, J. Watson, D. A. Siegel, D. C. Zacherl, and R. J. Toonen. 2010. Ocean currents help explain population genetic structure. Proc. R. Soc. Lond. B Biol. Sci. 20:695–706.

Yasuda, N., S. Nagai, M. Hamaguchi, K. Okaji, K. Gérard, and K. Nadaoka. 2009. Gene flow of *Acanthaster planci* (L.) in relation to ocean currents revealed by microsatellite analysis. Mol. Ecol. 18:1574–1590.

Abdullah et al., Genetic Diversity and Population Structure of Pronghorn Spiny Lobster *Panulirus penicillatus* in the Pacific Region, *Pacific Science* 68 (2), 2014

Appendix S1. Spatial Distribution of Control Region Haplotypes among *Panulirus penicillatus* from Eight Localities in the Western Pacific and Two Localities in the Eastern Pacific Region

	Locality									
Haplotype No.	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
1	1									
2	1									
3	1									
4	1									
5	1									
6	1									
7	1									
8	1									
9	1									
10	1									
11	1									
12	1									
13	1									
14	1									
15	1									
16	1									
17	1									
18	1									
19	1									
20	1									
21	1									
22	1									
23	1									
24	1									
25	1									
26	1									
27	1									
28	1									
29	1									
30	1									
31	1		1							
32	1									
33	1									
34	1									
35	1									
36	1									
37	1									
38	1									
39	1									
40	1				1					
41	1									
42	1									

Haplotype No.	Locality									
	Hachijiojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
43	1									
44	1									
45	1									
46		1								
47		1								
48		1								
49		1								
50		1								
51		1								
52		1								
53		1								
54		1								
55		1								
56		1								
57		1								
58		1								
59		1								
60		1								
61		1								
62		1								
63		1								
64		1								
65		1								
66		1								
67		1								
68		1								
69		1								
70		1								
71		1								
72		2								
73		1								
74		1								
75		1								
76		1								
77		1								
78		1								
79		1								
80		2								
81		1								
82		1								
83		1								
84		1								
85		1								

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
86		1								
87		1								
88		1								
89		1								
90		1								
91		1								
92		1								
93		1								
94			1							
95			1							
96			1							
97			1							
98			1	1						
99			1							
100			1							
101			1							
102			1							
103			1							
104			1							
105			1							
106			1							
107			1							
108	2		1							
109	1									
110	1									
111	1									
112	1									
113	1									
114	1									
115	1									
116	1									
117	1									
118	1									
119	1									
120	1									
121	1									
122	1									
123	1									
124	1									
125	1									
126	1									
127	1									
128	1									
129	1									

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
130			1							
131			1							
132			1							
133			1							
134			1							
135			1							
136			1							
137			1							
138			1							
139			1							
140			1							
141			1							
142			1							
143			1							
144			1							
145			1							
146			1							
147			1							
148			1							
149			1							
150			1							
151				1						
152				1						
153				1						
154				1						
155				1						
156				1						
157				1						
158				1						
159				1						
160				1						
161				1						
162				1						
163				1						
164				1						
165				1						
166				1						
167				1						
168				1						
169				1						
170				1						
171				1						
172				1						
173				1						

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
174				1						
175				1						
176				1						
177				1						
178				1						
179				1						
180				1						
181				1						
182				1						
183				1						
184				1						
185				1						
186				1						
187				1						
188				1						
189				1						
190				1						
191				1						
192				1						
193				1						
194				1						
195				1						
196				1						
197				1						
198				1						
199				1						
200				1						
201					1					
202					1					
203					1					
204					1					
205					1					
206					1					
207					1					
208					1					
209					1					
210					1					
211					1					
212					1					
213					1					
214					1					
215					1					
216					1					
217					1					

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
218					1					
219					1					
220					1					
221					1					
222					1					
223					1					
224					1					
225					1					
226					1					
227					1					
228					1					
229					1					
230					1					
231					1					
232					1					
233					1					
234					1					
235					1					
236					1					
237					1					
238					1					
239					1					
240	1									
241	1									
242	1									
243	1									
244	1									
245	1									
246	1									
247	1									
248		1								
249		1								
250		1								
251		1								
252		1								
253		1								
254		1								
255		1								
256		1								
257		1								
258		1								
259		1								
260		1								
261		1								

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
262						1				
263						1				
264						1				
265						2				
266						1				
267						1				
268						1				
269						1				
270						1				
271						1				
272						1				
273						1				
274						1				
275						1				
276						1				
277						1				
278						1				
279						1				
280						1				
281						1				
282						1				
283						1				
284						1				
285						1				
286						1				
287						1				
288						1				
289						1				
290						1				
291						1				
292						1				
293						1				
294						1				
295						1				
296						1				
297						1				
298						1				
299						1				
300						1				
301							1			
302							1			
303							1			
304							1			
305							1			

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
306							1			
307							1	1		
308							1			
309							1			
310							1			
311							1			
312							1			
313							1			
314							1			
315							1			
316							1			
317							1			
318							1			
319							1			
320							1			
321							1			
322							1			
323							1			
324							1			
325							1			
326							1			
327							1			
328							1			
329							1			
330							1			
331							1			
332							1			
333							1			
334							1			
335							1			
336							1			
337							1			
338							1			
339							1			
340							1			
341							1			
342							1			
343							1			
344								1		
345								1		
346								1		
347								1		
348								1		
349								1		

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
350								1		
351								1		
352								1		
353								1		
354								1		
355								1		
356								1		
357								1		
358								1		
359								1		
360								1		
361								1		
362								1		
363								1		
364								1		
365								1		
366								1		
367								1		
368								1		
369								1		
370								1		
371								1		
372	1									
373	1									
374	1									
375	1									
376	1									
377	1									
378	1									
379	1									
380	1									
381	1									
382	1									
383	1									
384	1									
385	1									
386	1									
387	1									
388	1									
389	1									
390									1	
391									1	
392									1	
393									1	

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
394									1	
395									1	
396									1	
397									1	
398									1	
399									1	
400									1	
401									1	
402									1	
403									1	
404									1	
405									1	
406									1	
407									1	
408									1	
409									1	
410									1	
411									1	
412									1	
413									1	
414									1	
415									1	
416									1	
417									1	
418									1	
419									1	
420									1	
421									1	
422									1	
423									1	
424									1	
425									1	
426									1	
427									1	
428									1	
429									1	
430									1	
431									1	
432									1	
433									1	
434									1	
435									1	
436									1	
437									1	

Haplotype No.	Locality									
	Hachijojima	Amamioshima	Okinawajima	Ishigakijima	Taiwan	Java Sea	Gabe Island	French Polynesia	Western sea of the Galápagos Islands	Isabela Island
438										1
439										1
440										2
441										1
442										1
443										1
444										1
445										1
446										1
447										1
448										1
449										1
450										1
451										1
452										1
453										1
454										1
455										1
456										1
457										1
458										1
459										1
460										1
461										1
462										1
463										1
464										1
465										1
466										1
467										1
468										1
469										1