Genetic diversity and population structure of pronghorn spiny lobster *Panulirus penicillatus* in the Pacific region

By Muhamad Fadry Abdullah*, Seinen Chow, Mitsuo Sakai, Jin-Hua Cheng and Hideyuki Imai

**Abstract**

Adult specimens of *Panulirus penicillatus* were collected from localities distributed in Japan: Hachijo Island, Ryukyu Archipelago (Amamioshima, Okinawajima, Ishigakijima); Taiwan: Taitung; Indonesia: Java Sea and Gebe Island; French Polynesia: Moorea Island; and Ecuador: Isabela Island. Phyllosoma larvae specimens were collected from the Western Sea of the Galápagos Islands. In total, 569-570 nucleotide sequences were analyzed from the mtDNA control region. Almost all individuals had a unique haplotype. Intrapopulation haplotype (*h*) and nucleotide (*π*) diversities were high for each locality, ranging from *h* = 0.9996–1.0000 and *π* = 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 Western/Central Pacific region, probably resulting from an extended planktonic larval stage and prevailing ocean currents. The present study detected genetic structure between Eastern Pacific populations and Western Pacific populations, indicating that despite the nearly 1-year larval period for this species, larvae generally do not pass over the East Pacific Barrier.

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Introduction

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, while it is also import as a fisheries commodity (Holthuis 1991, Ptacek et al. 2001, Abdullah et al. 2010). The species are found generally along the continental coast 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 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 considerable fisheries in tropical Pacific regions. The main total harvest of lobsters for the locals of the Pacific island nations is important, both economically and socially, despite the relatively small amount of reported production in worldwide terms (FAO 1987, Pitcher 1993). The recorded catch of the islands substantially underestimates the actual catch, primarily because a large proportion is consumed non-commercially. 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 in many author (eg. Carvalho and Hauser 1995 and references therein; Thorpe et al. 2000). The common supposition is that within a stock, there should be genetic homogeneity, while 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 distribute 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 significantly correlated with dispersal distance, but there were many exceptions, larval behavior can play a crucial role in determining dispersal distance (Shank 2009), type of eggs and life-history parameters are also significant 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 in natural condition to be >7–8 months. Minagawa (1990) cultured phyllosoma of *P. penicillatus* under laboratory conditions and described the phases from hatching up to the mid-stages (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). As 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. 2006a, b). Further sequence analysis of cytochrome oxidase I and 16S rDNA from a Pacific population of *P. penicillatus* indicated two distinct sub-populations 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 this study, we analysed 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 were 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 (Table 1) throughout the Western/Central Pacific and Eastern Pacific Ocean (Fig. 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 larvae samples of *P. penicillatus* from the Western Sea of the Galápagos Islands of the East Pacific Ocean were provided 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 38°C for 3 hours and then genomic DNA was extracted using phenol-chloroform procedure and precipitated with absolute ethanol according 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 *Panulirus*12s primer (5′-TATAGCAAGAATCAAACATATAG), in the 12S ribosomal RNA gene, and the *Panulirus*-tRNA primer (5′-CATAGACGGGTATGAGCCCGT), in the tRNA\textsuperscript{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 *Panulirus*12s and *Panulirus*-tRNA. Primers *Panulirus*12s 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 s at 95°C, 30 s 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 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 $\Phi_{ST}$ (using genetic distance). $\Phi_{ST}$ 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 $\Phi_{ST}$ value was tested by 1000 permutation. 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 $\Phi_{ST}$ being relatively unaffected by high allelic diversity, unlike $F_{ST}$ (Bird et al. 2011), we only report $\Phi_{ST}$ values. A neighbour-joining tree (Saitou and Nei 1987) based on $\Phi_{ST}$ 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 ($\tau, \theta_0, \theta_1$) 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 are commonly used to test neutrality; however, they 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 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, while the other two were shared only between individuals restricted to the same geographical site (Supplementary Appendix S1, available online).

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); Amamiohshima, Okinawajima, and Ishigakijima Island 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). Additionally,
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, ($F_{CT} = 0.76, P < 0.05$). An AMOVA based on conventional F-statistics (haplotype frequency only) failed to detect any population structure, while an AMOVA using the Tamura-Nei (1993) distance performed on the mtDNA sequence data set revealed a structure in population. There was no significant population distinction within samples from Western/Central Pacific populations by AMOVA. The Pairwise $\Phi_{ST}$ among populations were high between Eastern Pacific and Western/Central Pacific population (0.858–0.889) (Table 2). Congruent with the pattern observed in the network haplotypes (Fig.3), 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 sub-structure 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 $\Phi_{ST}$ among population was small (%var -0.19 to -0.11) and did not significantly differ from 0 (Table 3). A neighbour-joining tree based on $\Phi_{ST}$ values showed a prominent genetic break between Western/Central Pacific and Eastern Pacific populations (Fig.2). Based on the goodness-of-fit test, *P. penicillatus* could be fitted to an expansion model (all the population has $P > 0.05$) (Fig.4). 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 = 0.039). Appropriate dating of population expansions was not possible because mutation rates for the mtDNA control region in this species have not been estimated. Considering this, the mutation rate was calculated according to Babbucci et al. (2010), ranging between 8.1-13.4% per MYR were applied to the model $T = \tau / 2u$ (Rogers and Harpending 1992), where $T =$ time since expansion, $2u = \mu \times$ number bases sequenced $\times$ generation time (3 yr generation). A rough estimation suggested that population expansions occurred in the late Pleistocene: 52,000 to 200,000 years ago for Western/Central Pacific $P. penicillatus$, and 41,000 to 68,000 years 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 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 variation in genetic 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 divergence was also observed between Brazilian and Western 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 4500 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*; secondary 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 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 present study, the mtDNA control region exhibited high genetic variability (*h* = 0.9996 to 1.0000 and *π* = 0.0300 to 0.0581), which are relatively high values compared with the control region diversities of other *Panulirus* sp. [*P. inflatus*: *h* = 0.957 to 1.0000 and *π* = 0.019 to 0.028 (García-Rodríguez and Perez-Enriquez 2008); *P. argus*: *h* = 0.667 to 1.0000 and *π* = 0.003 to 0.066 (Diniz et al. 2005)]. The control region is a non-coding 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 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 (Garcia-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 year) of the phyllosoma (Johnson 1968, Booth and Phillips 1994, Matsuda 2006) coupled with the significant 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 along Northwestern Pacific. Furthermore, transportation of larvae between the Western-Central Pacific more likely occurred indirectly via stepping stones by the Equatorial Currents, and small amount of larval dispersal over years might result in genetic homogeneity. Unfortunately, population analysis within Eastern Pacific could not be done due to having 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 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).

The pronghorn spiny lobster, *P. penicillatus*, has been reported to be commercially abundant in the Eastern Pacific, particularly the Galápagos Islands (Holthuis and Loesch 1967, George and Main 1967). *P. 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 counter currents within the Equatorial Current System (Fig. 1) could provide possible routes for the return of Eastern Pacific species that have either fortuitously or instinctively shifted into counter currents, 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. The present study detected genetic structure between Eastern Pacific population and Western/Central Pacific populations, indicating that despite the nearly 1-year larval period for this species (e.g., Booth and Phillips 1994, George 2005), could the larvae generally not pass over the East Pacific Barrier, or they could regularly cross this barrier but fail to survive in appreciable number in other region due to some selective advantage.
of local recruits and habitat preferences. The absence of intermediate ‘steppingstones’— 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 significant barrier to dispersal and gene flow (Crandall et al. 2012). More importantly, the lack of shared mtDNA haplotypes and large $\Phi_{ST}$ 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 since 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 modelled. 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 behaviour such as vertical migration, together with a retentive oceanographic environment, may increase the potential for self-recruitment (Butler 2011).

Adult *P. penicillatus* show variations in body colour, 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 owing to equatorial currents. Consistent with previous findings, we identified morphological variability in the body colour of *P. penicillatus* species: Eastern Pacific populations had a reddish body and Western/Central Pacific populations had a greenish body (Fig. 3). George (2006) proposed that
the genetic distinctions for subpopulations of *P. penicillatus* from the Eastern Pacific, referred as *P. penicillatus* red, could be explained by assuming that the East Pacific Barrier of Ekman (1953) represents a significant barrier that maintains the isolation of these subpopulations. However, further research is recommended to clearly establish the correlation in the variation of morphological body colour 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 years ago for Western/Central Pacific and 41,000 to 68,000 years ago for the Eastern Pacific during the Pleistocene. The late Pleistocene Period (the past one million years) was punctuated by a series of large glacial and interglacial changes (Imbrie et al. 1992).

The pattern of mismatch distributions of *P. penicillatus* differed between 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, while the Eastern Pacific population is smaller and probably more recent in origin.

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Table 1 Sample locality, sample abbreviation (ID), sample size (N), year of collection, intrapopulation haplotype diversity (h) and nucleotide diversity (π) for *Pandalus ocineratus* mitochondrial DNA control region. Populations were binned from sampling locality to six group of population defined by geographical region and oceanographic history. See text for details.

<table>
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<th>Sample ID</th>
<th>Locality</th>
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<th>year</th>
<th>Population</th>
<th>Number of haplotypes</th>
<th>h</th>
<th>π</th>
<th>Population region</th>
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<td>FP</td>
<td>Moorea, French Polynesia</td>
<td>47</td>
<td>2011</td>
<td>French Polynesia</td>
<td>47</td>
<td>1.0000 ± 0.0044</td>
<td>0.0498 ± 0.0246</td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td>Isabela Island, Ecuador</td>
<td>48</td>
<td>2010</td>
<td>East Pacific</td>
<td>80</td>
<td>0.9997 ± 0.0020</td>
<td>0.0300 ± 0.0150</td>
<td>Eastern Pacific</td>
</tr>
<tr>
<td>EP*</td>
<td>Western Sea of the Galápagos Islands, Ecuador</td>
<td>33</td>
<td>2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>480</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Phyllosoma larvae samples were provided by Chow et al. (2011).*
<table>
<thead>
<tr>
<th></th>
<th>Hachijojima Island</th>
<th>Ryukyu Archipelago</th>
<th>Taiwan</th>
<th>Indonesia</th>
<th>French Polynesia</th>
<th>East Pacific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hachijojima Island</td>
<td>—</td>
<td>0.00159</td>
<td>0.01011</td>
<td>0.00094</td>
<td>0.00944</td>
<td>0.88118</td>
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<tr>
<td>Ryukyu Archipelago</td>
<td>0.21622</td>
<td>—</td>
<td>-0.00430</td>
<td>-0.00005</td>
<td>-0.00338</td>
<td>0.85811</td>
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<tr>
<td>Taiwan</td>
<td>0.1081</td>
<td>0.85586</td>
<td>—</td>
<td>-0.00121</td>
<td>-0.00966</td>
<td>0.88506</td>
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<tr>
<td>Indonesia</td>
<td>0.36036</td>
<td>0.34234</td>
<td>0.59459</td>
<td>—</td>
<td>0.00075</td>
<td>0.87229</td>
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<tr>
<td>French Polynesia</td>
<td>0.16216</td>
<td>0.78378</td>
<td>0.95495</td>
<td>0.34234</td>
<td>—</td>
<td>0.88886</td>
</tr>
<tr>
<td>East Pacific</td>
<td>0.00000*</td>
<td>0.00000*</td>
<td>0.00000*</td>
<td>0.00000*</td>
<td>0.00000*</td>
<td>—</td>
</tr>
</tbody>
</table>

*P<0.05
Table 3 Amova results showing degree of freedom (df), variance components (Var.), percent variation (% Var.) and \( \Phi \)-statistics for *Panulirus penicillatus*. Runs included all populations as described in the text (1000 permutations).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Var</th>
<th>% Var</th>
<th>( \Phi )-statistics</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All sites (Western-Central/Eastern Pacific)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among regions</td>
<td>1</td>
<td>84.19733</td>
<td>85.23</td>
<td>( \Phi_{CT} = 0.85226 )</td>
<td>0.16129</td>
</tr>
<tr>
<td>Among populations</td>
<td>4</td>
<td>0.00997</td>
<td>0.01</td>
<td>( \Phi_{SC} = 0.00068 )</td>
<td>0.51222</td>
</tr>
<tr>
<td>Within populations</td>
<td>474</td>
<td>14.58564</td>
<td>14.76</td>
<td>( \Phi_{ST} = 0.85236 )</td>
<td>0.00000</td>
</tr>
<tr>
<td><strong>Western-Central Pacific Region</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Hachijo-jima-Ryukyu-Taiwan-Indonesia/French Polynesia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among regions</td>
<td>1</td>
<td>-0.0303</td>
<td>-0.19</td>
<td>( \Phi_{CT} = -0.00193 )</td>
<td>0.78495</td>
</tr>
<tr>
<td>Among populations</td>
<td>3</td>
<td>0.00192</td>
<td>0.01</td>
<td>( \Phi_{SC} = 0.00012 )</td>
<td>0.43304</td>
</tr>
<tr>
<td>Within populations</td>
<td>394</td>
<td>15.75562</td>
<td>100.18</td>
<td>( \Phi_{ST} = -0.00181 )</td>
<td>0.49853</td>
</tr>
<tr>
<td>(Hachijo-jima-Ryukyu-Taiwan/Indonesia-French Polynesia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among regions</td>
<td>1</td>
<td>-0.01748</td>
<td>-0.11</td>
<td>( \Phi_{CT} = -0.00111 )</td>
<td>0.70283</td>
</tr>
<tr>
<td>Among populations</td>
<td>3</td>
<td>0.00435</td>
<td>0.03</td>
<td>( \Phi_{SC} = 0.00028 )</td>
<td>0.44673</td>
</tr>
<tr>
<td>Within populations</td>
<td>394</td>
<td>15.75562</td>
<td>100.08</td>
<td>( \Phi_{ST} = -0.00083 )</td>
<td>0.50538</td>
</tr>
<tr>
<td>(Hachijo-jima-Ryukyu-Taiwan/Indonesia/French Polynesia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among regions</td>
<td>2</td>
<td>-0.02017</td>
<td>-0.13</td>
<td>( \Phi_{CT} = -0.00128 )</td>
<td>0.47019</td>
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<tr>
<td>Among populations</td>
<td>2</td>
<td>0.00763</td>
<td>0.05</td>
<td>( \Phi_{SC} = -0.00080 )</td>
<td>0.44477</td>
</tr>
<tr>
<td>Within populations</td>
<td>394</td>
<td>15.75562</td>
<td>100.08</td>
<td>( \Phi_{ST} = -0.00080 )</td>
<td>0.52395</td>
</tr>
</tbody>
</table>
Fig. 1 Map of the Pacific Ocean showing warm (solid lines) and cold currents (dashed lines), including the Oyashio Current (OY), 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). Dash-rectangles indicate grouping population (i.e Ryukyu Archipelago, Indonesia and East Pacific), closed circles indicate Adult samples and triangle refer to phyllosoma samples. Sampling site abbreviations correspond to Table 1. Ocean current was drawn referring to Colling (2001).
Fig. 2 Neighbor-joining population tree based on $\Phi_{ST}$ 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 Western/Central and Eastern was calculated using SAMOVA. The results suggest that there is high gene flow between populations belonging to the Western/Central Pacific region, whereas restricted gene flow found between populations of the Western/Central Pacific region and Eastern Pacific region of *Panulirus penicillatus*. The Japanese spiny lobster *P. japonicus* was used as outgroup.
Fig. 3. *Panulirus penicillatus* body color and haplotype network for individuals sampled from the Pacific region. Sizes of the circles are proportional to the frequency of each haplotype; lengths of the lines are relative to the number of mutations between haplotypes.
Fig. 4 Mismatch distribution established for *Panulirus penicillatus* mitochondrial control region. The bars represent the observed frequency of the pairwise differences among haplotypes, while 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; $\theta_0$, pre-expansion and $\theta_1$, post-expansion population sizes; $\tau$ time in number of generations, elapsed since the sudden expansion episode. Tajima’s (1989) D test value and its statistical significance are also given.
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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.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Locality</th>
<th>Confidence Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table above shows the spatial distribution of control region haplotypes among Panulirus penicillatus from eight localities in the western Pacific and two localities in the eastern Pacific region.