Morphological differences among phyllosoma larvae of the pronghorn spiny lobster, *Panulirus penicillatus*, from the western, central, and eastern areas of the Pacific Ocean

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**Abstract.**—The pronghorn spiny lobster, *Panulirus penicillatus*, is known to have the widest distribution among palinurid lobster species, occurring in tropical and subtropical areas of the Indo-Pacific Ocean. In the Pacific Ocean, mitochondrial DNA analyses have revealed that the western–central and eastern populations are genetically isolated. We performed morphological investigations of mid- to late-stage phyllosoma larvae collected in western, central, and eastern areas of the Pacific Ocean. The larvae from the western area had a significantly longer cephalic shield, shorter abdomen, and longer eyestalk than those from the eastern area. Additionally, for larger larvae, the widest part of the cephalic shield in larvae from the western area was located closer to the middle of the median line of the cephalic shield than in larvae from the eastern area. The ratio of width to length of cephalic shield and the ratio of cephalic shield width to thorax width are key traits for distinguishing between the larvae from the western and eastern areas. The morphology of larvae from the central area tended to be similar to that of larvae from the western area.

**Key words:** phyllosoma, body shape, geographic variation, metric character, relative growth, *Panulirus penicillatus*, Pacific Ocean

**Introduction**

The pronghorn spiny lobster, *Panulirus penicillatus*, occurs in tropical and subtropical areas of the Indo-Pacific Ocean. Its distribution is the widest among all species of spiny lobster, as there is no other spiny lobster species distributed across the Pacific Ocean (Holthuis 1991). The pronghorn spiny lobster is found in shallow water, usually at depths of less than 4 m, on rocky surfaces on the outer slopes of reefs and in water channels (Cockcroft et al. 2011). This lobster is an important target for coastal fisheries and supports the economy of many countries (Munro 2000). In many parts of its range, however, this species appears to be declining as a result of intense fishing pressure (Cockcroft et al. 2011). For the purpose of determining a sustainable catch for this lobster, it is necessary to perform adequate stock assessment and implement a fisheries management plan based on an understanding of the population structure.

Recently, mitochondrial DNA sequence analysis has been used to investigate the population structure of this lobster species in the Indo-Pacific Ocean (Chow et al. 2011, Abdullah et al. 2014a, b, Inacchei et al. 2016). *Panulirus penicillatus* in the Pacific Ocean has been divided into two distinct populations—the western–central population and the eastern population—based on phylogenetic analyses of adult and phyllosoma larval samples collected throughout the Pacific Ocean (Chow et al. 2011, Abdullah et al. 2014a, Inacchei et al. 2016). Although this lobster has a long-lived teleplan-
ic larval phase of at least 7–8 months, like many palinurid lobsters (Johnson 1968, Matsuda et al. 2006), the vast expanse of the Pacific Ocean with no islands and no shallow substrate known as the East Pacific Barrier (EPB) appears to have effectively isolated these two populations. To date no notable differences have been found between the western–central and eastern populations in form or configuration of the body or appendages of adult lobsters, but individuals of the two populations have different body colors: greenish in the western–central population and reddish brown in the eastern population (George 2006, Abdulalah et al. 2014a).

In the present study, we compared the body shape of *P. penicillatus* larvae collected in the western, central, and eastern Pacific Ocean. This is the first report on regional differentiation of larval morphology in this spiny lobster species.

### Materials and Methods

**Collection of phyllosoma larvae**

Phyllosoma samples for morphological examination were collected in three areas—the western Pacific (WP: station numbers 1–5 in Fig. 1 and Table 1), the central Pacific (CP: station 6), and the eastern Pacific (EP: station 7)—from the research vessels of the Fishery Agency of Japan and the Japan Fisheries Research and Education Agency from 2004 to 2016. Samples were collected by using an Isaacs-Kidd midwater trawl (8.7-m² opening, 13 m long, 6-mm mesh, canvas cod-end) (Isaacs and Kidd 1953), a Matsuda-Oozeki-Hu midwater trawl (5-m² opening, 12 m long, 1.59-mm mesh, cod-end bucket) (Oozeki et al. 2004), a large midwater trawl (NST-660-SR, Nichimo Co. Ltd., Tokyo, Japan; 161 m long, 10.4-mm to 60-mm mesh, 14.5-mm cod-end), or a “larva capture” net (LC-100²-R3, Nichimo Co. Ltd.; 100-m² opening, 36 m long, 6-mm mesh, cod-end bucket) (Table 1). The towing velocity was 1–2.5 knots (kn) for the Isaacs-Kidd midwater trawl, 4–5 kn for the Matsuda-Oozeki-Hu midwater trawl and midwater trawl, and 1–2 kn for the larva capture net (1 kn = 1.852 km/h). Phyllosoma larvae sorted from the cod-end samples were preserved in 70% ethanol and transferred to the laboratory.

![Fig. 1. Collecting locations (open circles) for the phyllosoma larvae of *Panulirus penicillatus* in the Pacific Ocean. See Table 1 for detailed information.](image-url)
Identification of phyllosoma larvae

Phyllosoma larvae that were potentially *P. penicillatus* based on appearance were sorted out from the preserved samples using the morphological features reported by Chow et al. (2006a) and Matsuda et al. (2006). The larval specimens selected on the basis of morphology were subjected to genetic analysis using nucleotide sequence or restriction fragment length polymorphism, as previously described by Chow et al. (2006a, b). Larvae determined to be *P. penicillatus* from the DNA analysis were used for subsequent examination.

Phyllosoma measurements and habitus analysis

For examinations of body habitus, *P. penicillatus* larvae from the three areas were measured using a Nikon profile projector (model V-12A, Nikon Ltd., Tokyo, Japan) as follows: body length (BL), from the anterior margin of the cephalic shield between the eyestalks to the posterior end of the abdomen; cephalic shield length (CL), from the anterior margin between the eyestalks to the posterior margin of the cephalic shield; cephalic shield width (CW), measured at the widest part of the cephalic shield; the length between the anterior margin and the widest part of the cephalic shield (LBAW), measured along the midline of the cephalic shield from the anterior margin of the cephalic shield to the point of intersection between the lines giving CL and CW; thorax width (TW), measured at the widest part of the thorax; abdominal length (AL), from a line level with the base of the abdomen to the posterior end of the abdomen; and eyestalk length (ESL), from the anterior margin of the cephalic shield to the base of the eye (Fig. 2).

To examine shifts in body shape with larval development, we analyzed allometric relationships between BL and body dimensions (CL, CW, TW, AL, and ESL) on the assumption that the relationships could be expressed with a logistic equation as follows:

\[
G_i = \frac{a_i}{1 + \exp\left(-b_i(BL - c_i)\right)},
\]

where \(G_i\) is the value of the \(i\)th larval body dimension (\(i = 1\) for CL, \(i = 2\) for CW, \(i = 3\) for TW, \(i = 4\) for AL, and \(i = 5\) for ESL), and \(a_i\), \(b_i\), and \(c_i\) are parameters for the \(i\)th body dimension (to be estimated). A normal distribution \(N(\bar{G}_i, \sigma_i^2)\) was assumed for describing the random error of each body dimension.
dimension, where $\hat{G}_j$ is the estimated $i$th body dimension for the $j$th datum of $BL$, and $\hat{\sigma}_j$ is the standard deviation. We assumed that $\hat{\sigma}_j$ is proportional to the following linear function of $BL_j$:

$$\sigma_j = d_i + e_i BL_j,$$

where $d_i$ and $e_i$ are parameters for adjusting the degree of the effect of $BL_j$. The range from $\hat{G}_j - 1.96 \hat{\sigma}_j$ to $\hat{G}_j + 1.96 \hat{\sigma}_j$ represents the estimated 95% confidence interval.

The parameters in equations (1) and (2) were estimated for each body dimension ($i = 1$ to 5) by maximizing the following log-likelihood function with the aid of nonlinear optimization software:

$$\mathcal{D}_i = -\sum_{j=1}^{n} \left( \frac{1}{2} \ln 2\pi + \ln \hat{\sigma}_j \right) - \sum_{j=1}^{n} \frac{(G_j - \hat{G}_j)^2}{2\hat{\sigma}_j^2}.$$  

(3)

The relationships between $BL$ and five body-dimension ratios ($CW : CL$, $CW : TW$, $LBAW : CL$, $AL : BL$, and $ESL : CL$) representing the characteristics of body shape were assumed to be expressed by a first-order equation as follows:

$$R_k = f_k + (g_k - f_k) \{1 - \exp \left(-h_k BL\right)\},$$

(4)

where $R_k$ is the value of the $k$th body-dimension ratio ($k = 1$ for $CW : CL$, $k = 2$ for $CW : TW$, $k = 3$ for $LBAW : CL$, $k = 4$ for $AL : BL$, and $k = 5$ for $ESL : CL$), and $f_k$, $g_k$, and $h_k$ are parameters for the $k$th body-dimension ratio (to be estimated). A normal distribution $N(\hat{R}_{jk}, \hat{\sigma}_k^2)$ was assumed for describing the random error of each ratio, where $\hat{R}_{jk}$ is the estimated $k$th body-dimension ratio for the $j$th datum of $BL$, and $\hat{\sigma}_k$ is the standard deviation. We assumed that $\sigma_k$ is constant and independent of $BL_j$. The range from $\hat{R}_{jk} - 1.96 \hat{\sigma}_k$ to $\hat{R}_{jk} + 1.96 \hat{\sigma}_k$ represents the estimated 95% confidence interval.

The parameters in equation (4) and $\sigma_k$ were estimated for each body-dimension ratio ($k = 1$ to 5) by maximizing the following log-likelihood function with the aid of nonlinear optimization software:

$$\mathcal{D}_k = -\sum_{j=1}^{n} \left( \frac{1}{2} \ln 2\pi + \ln \hat{\sigma}_k \right) - \sum_{j=1}^{n} \frac{(R_{jk} - \hat{R}_{jk})^2}{2\hat{\sigma}_k^2}.$$  

(5)

Developmental stages

The larvae from the WP, CP, and EP were separated into developmental stages on the basis of staging criteria for cultured larvae of $P. penicillatus$ (Matsuda et al. 2006). The
body dimensions (BL, CL, CW, TW, AL, and ESL) for the larvae from the three areas were averaged for each morphological stage. The body-dimension ratios (CW : CL, CW : TW, LBAW : CL, AL : BL, and ESL : CL) for each stage of the larvae from the WP and EP were compared using a Mann–Whitney U test. The test was considered significant at $P < 0.05$. The number of larvae from the CP was small ($n = 2$), so the larvae from the CP were not subjected to the test.

## Results

The numbers of larvae determined to be *P. penicillatus* from morphologic features and subsequent genetic analysis were 80 from the WP, 2 from the CP, and 60 from the EP. Those from the WP and CP were determined to belong to the western–central population, and the EP specimens were from the eastern population.

### Changes in body shape with development

The 80 larvae from the WP and the 60 larvae from the EP were separately subjected to the habitus analyses, whereas the 2 larvae from the CP were not. The values for CL, CW, TW, AL, and ESL in the larvae from the WP and EP increased in a slight sigmoid curve with development (Fig. 3). Estimated parameters for the logistic equation for each body dimension are given in Table 2. The fitted lines that represent changes in the mean values of body dimensions as a function of BL show similar trends for CW and TW of both the WP and EP larvae. On the other hand, CL and ESL of the WP larvae tend to be longer than in the EP larvae, and AL of the WP larvae was smaller than that of the EP larvae. The CP larvae (15.8-mm BL and 38.8-mm BL) tend to have values intermediate between the WP and EP larvae.

The differences in body shape between larvae from the WP and EP were more conspicuous when body-dimension ratios (CW : CL, CW : TW, LBAW : CL, AL : BL, and ESL : CL) were compared (Fig. 4). Estimated parameters for the first-order equation for each body-dimension ratio are given in Table 3. The CW : CL and CW : TW ratios were distinct between the larvae from the WP and EP; CW : CL and CW : TW of the WP larvae were lower than those of the EP larvae over the entire BL range (10–40 mm) examined in this study. There was no overlap in the 95% confidence intervals of CW : CL for the larvae from the two areas between 18-mm BL and 25-mm BL. The mean values of CW : CL and CW:TW for the WP larvae over 30-mm BL were estimated as 0.73 and 1.00, respectively, and those of the EP larvae were 0.79 and 1.06. In the larvae from the WP, the values of LBAW : CL decreased from 0.53 at 10-mm BL to 0.49 at 20-mm BL, and remained constant at 0.49 thereafter. In the larvae from the EP, on the other hand, the values of LBAW : CL consistently decreased with development; the values were 0.51 at 10-mm BL, 0.48 at 20-mm BL, and 0.45 at 35-mm BL. These results indicate that the position of the widest part of the cephalic shield in the WP larvae is close to the midpoint of the median line of the cephalic shield, and that in the developed EP larvae it is located more anteriorly. The AL : BL values of the WP larvae were significantly lower than those of the EP larvae for larvae larger than 31-mm BL, as there is no overlap in the 95% confidence intervals between the WP and EP larvae. There was a trend of higher ESL : CL values in the WP larvae than in the EP larvae, although there is considerable overlap in the 95% confidence intervals of the larvae from the two areas. The CP larvae had values of CW : TW, LBAW : CL, and ESL : CL within the 95% confidence intervals of both the WP and EP larvae, except for the values of CW : TW and LBAW : CL in the larva with 15.8-mm BL. On the other hand, the values of CW : CL and AL : BL for the CP larvae were within the 95% confidence intervals of the WP larvae.
Fig. 3. Allometric relationships between body length (BL) and body dimensions (CL, cephalic shield length; CW, cephalic shield width; TW, thorax width; AL, abdomen length; ESL, eyestalk length) in the phyllosoma larvae of *Panulirus penicillatus* from the western (WP), central (CP), and eastern (EP) areas of the Pacific Ocean. The thin and thick solid lines show the estimated mean values for the larvae from the WP and EP, respectively. The 95% confidence intervals for respective mean values are shown by thin and thick dashed lines. Solid circles represent data for the WP larvae, open circles represent data for the EP larvae, and open triangles represent data for the CP larvae.
BODY SHAPE VARIATION IN _PANULIRUS PENICILLATUS_ LARVAE

Comparison of body dimensions of phyllosoma larvae from the WP, CP, and EP by developmental stage

The 80 larvae from the WP were assigned into five stages, from Stage VI to Stage X, the 2 larvae from the CP were assigned into Stage VII and Stage X, respectively, and the 60 larvae from the EP were assigned into four stages, from Stage VII to Stage X. The various body dimensions for each stage are presented in Table 4. There were overlaps between the larvae from the WP and EP in the range of each body dimension at all stages, with a tendency for BL, CL, and ESL to be higher in the WP larvae than in the EP larvae.

The WP larvae had values of CW : CL and CW : TW that were significantly lower than the EP larvae in almost all stages, indicating that the cephalic shield of the WP larvae was relatively narrower than that of the EP larvae (Fig. 5, Table 5). The mean value for LBAW : CL in Stage X of the WP larvae was 0.495 and significantly higher than that of the EP larvae (0.453), also indicating that the widest part of the cephalic shield in the WP larvae is significantly closer to the central point of the median line of the cephalic shield than that in the EP larvae, which is located more anteriorly. There was a trend of shorter abdomens and longer eyestalks in the WP larvae than in the EP larvae, as reflected by AL : BL and ESL : CL. The Stage VII CP larva had body-dimension ratios closer to those of WP larvae than to those of EP larvae; body-dimension ratios in the Stage X CP larva tended to be intermediate between the WP and EP larvae.

Discussion

The CW : TW ratio has been a key trait for identifying phyllosoma larvae of four species-groups of _Panulirus_ lobsters (Groups I–IV), as proposed by George and Main (1967), and even species within the same group (McWilliam 1995, Chow _et al._ 2006a). Mid- to final-stage phyllosoma larvae of _P. penicillatus_ belonging to Group II had significantly higher CW : TW values than those of Group I lobsters and lower CW : TW values than those of Group IV lobsters (Chow _et al._ 2006a). The present study confirms that the CW : TW ratio is even applicable for distinguishing _P. penicillatus_ larvae from the western and eastern areas of the Pacific Ocean (WP and EP). The CW : CL ratio is also valid for separating the larvae from the two areas, because there was little overlap in the 95% confidence intervals of this ratio between the larvae from the two areas of the Pacific Ocean. See text for explanation of each parameter.

Table 2. Estimated parameter values for the logistic equation \( G = a(1 + \exp\{-b(CL - c)\}) \) and the standard deviation \( \sigma (\sigma = d + eBL) \) for the allometric relationships between body length (BL) and body dimensions (G) for the phyllosoma larvae of _Panulirus penicillatus_ from the western (W) and eastern (E) areas of the Pacific Ocean. See text for explanation of each parameter.

| Parameter | Body dimension/Area | | | | |
|-----------|---------------------|-----|-----|-----|-----|
|           | CL                  | CW  | TW  | AL  | ESL |
|           | W                   | E   | W   | E   | W   | E   |
| a         | 2.62                | 24.4| 18.8| 18.4| 19.4| 18.5| 16.0| 12.8| 8.6 |
| b         | 0.112               | 0.109| 0.126| 0.134| 0.111| 0.111| 0.132| 0.163| 0.125| 0.118 |
| c         | 17.5                | 16.2| 18.0| 16.2| 18.0| 16.9| 36.4| 30.2| 16.7|
| d         | 0.129               | 0.000| 0.124| 0.020| 0.147| 0.000| 0.000| 0.000| 0.000| 0.001|
| e         | 0.0118              | 0.0076| 0.0116| 0.0086| 0.0077| 0.0090| 0.0116| 0.0043| 0.0126| 0.0115|

CL, cephalic shield length; CW, cephalic shield width; TW, thorax width; AL, abdomen length; ESL, eyestalk length
Fig. 4. Relationships between body length (BL) and five body-dimension ratios (CW : CL, CW : TW, LBAW : CL, AL : BL, and ESL : CL) in the phyllosoma larvae of Panulirus penicillatus from the western (WP), central (CP), and eastern (EP) areas of the Pacific Ocean. Thin and thick solid lines show estimated mean values for larvae from the WP and EP, respectively. The 95% confidence intervals for the respective mean values are shown by thin and thick dashed lines. Solid circles represent data for the WP larvae, open circles represent data for the EP larvae, and open triangles represent data for the CP larvae. CW, cephalic shield width; CL, cephalic shield length; TW, thorax width; LBAW, length between the anterior margin and the widest part of the cephalic shield; AL, abdomen length; ESL, eyestalk length.
areas for larvae larger than 18-mm BL. Although there were only two larvae from the central area of the Pacific Ocean (CP) in the present study and their morphologies did not clearly match the larvae from either the WP or EP, they tended to be more similar to the WP larvae. These observations indicate that the larvae of the two populations of *Panulirus penicillatus* in the Pacific Ocean (the western–central population and the eastern population) have different morphological characteristics.

Polygenetic analysis of mtDNA has con-

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**Table 3.** Estimated parameter values for the first-order equation \( R = f + (g - f) \{1 - \exp(-hBL)\} \) and the standard deviation \( \sigma \) for the relationships between body length (BL) and body-dimension ratios (\( R \)) for the phyllosoma larvae of *Panulirus penicillatus* from the western (W) and eastern (E) areas of the Pacific Ocean. See text for an explanation of each parameter.

| Parameter | Body-dimension ratio/Area |
|-----------|---------------------------|
|           | CW : CL | CW : TW | LBAW : CL | AL : BL | ESL : CL |
|           | W       | E       | W       | E       | W       | E       | W       | E       | W       | E       |
| \( f \)   | 0.365   | 0.000   | 0.549   | 0.000   | 1.316   | 0.552   | 0.006   | -0.030  | 0.341   | 0.009   |
| \( g \)   | 0.74    | 0.80    | 1.00    | 1.06    | 0.48    | 0.39    | -0.08   | -0.035  | 0.11    | 0.32    |
| \( h \)   | 0.1240  | 0.179   | 0.139   | 0.218   | 0.288   | 0.028   | -0.032  | -0.017  | 0.000   | 0.325   |
| \( \sigma \) | 0.0198  | 0.0145  | 0.0285  | 0.0201  | 0.0200  | 0.0136  | 0.0137  | 0.0055  | 0.0229  | 0.0155  |

CL, cephalic shield length; CW, cephalic shield width; TW, thorax width; LBAW, length between the anterior margin and the widest part of the cephalic shield; AL, abdomen length; ESL, eyestalk length.

**Table 4.** Comparison of morphometric sizes in Stage VI to X phyllosoma larvae of *Panulirus penicillatus* from the western (W), central (C), and eastern (E) areas of the Pacific Ocean. BL, body length; CL, cephalic shield length; CW, cephalic shield width; TW, thorax width; AL, abdomen length; ESL, eyestalk length.

| Stage/Area (No. of inds.) | VI W (10) | VII W (13) | C (1) E (48) | VIII W (25) | E (7) | IX W (22) | E (2) | X W (10) | C (1) E (3) |
|---------------------------|-----------|------------|--------------|-------------|------|-----------|------|---------|-------------|
| BL                        | Mean      | 11.1       | 15.3         | 15.8        | 15.1 | 23.0      | 19.3 | 29.6    | 26.9        | 39.0        | 38.8        | 36.8        |
|                           | Min.      | 7.8        | 11.1         | 12.8        | 17.5 | 17.7      | 24.2 | 26.1    | 35.0        | —            | 36.0        |
|                           | Max.      | 12.4       | 17.8         | —           | 17.7 | 28.1      | 22.4 | 32.8    | 27.8        | 42.6         | —            | 37.7        |
| CL                        | Mean      | 8.5        | 11.8         | 12.2        | 11.5 | 16.9      | 14.2 | 20.6    | 17.9        | 24.2         | 23.7         | 22.4        |
|                           | Min.      | 5.8        | 8.4          | —           | 9.9  | 13.4      | 13.3 | 17.7    | 17.5        | 21.5         | —            | 21.9        |
|                           | Max.      | 9.7        | 13.8         | —           | 13.5 | 19.6      | 16.0 | 22.6    | 18.4        | 26.4         | —            | 37.7        |
| CW                        | Mean      | 5.5        | 8.0          | 8.3         | 8.5  | 12.2      | 11.0 | 15.1    | 14.4        | 17.6         | 17.8         | 17.5        |
|                           | Min.      | 3.6        | 5.1          | —           | 7.1  | 9.3       | 10.2 | 13.1    | 13.8        | 15.9         | —            | 16.5        |
|                           | Max.      | 6.1        | 9.5          | —           | 10.2 | 15.0      | 12.7 | 16.6    | 15.1        | 19.2         | —            | 18.1        |
| TW                        | Mean      | 6.0        | 8.5          | 8.8         | 8.3  | 12.3      | 10.4 | 15.1    | 13.6        | 17.9         | 17.2         | 16.9        |
|                           | Min.      | 4.2        | 5.9          | —           | 7.1  | 9.7       | 9.7  | 13.0    | 13.1        | 16.4         | —            | 16.5        |
|                           | Max.      | 6.8        | 9.8          | —           | 9.7  | 14.6      | 11.9 | 16.6    | 14.1        | 19.2         | —            | 17.4        |
| AL                        | Mean      | 0.6        | 0.9          | 0.9         | 1.0  | 2.4       | 1.9  | 4.6     | 4.8         | 9.6          | 9.9          | 9.5         |
|                           | Min.      | 0.5        | 0.4          | —           | 0.7  | 1.2       | 1.6  | 2.8     | 4.4         | 8.6          | —            | 9.4         |
|                           | Max.      | 0.7        | 1.2          | —           | 2.3  | 3.6       | 2.5  | 5.8     | 5.1         | 10.4         | —            | 9.7         |
| ESL                       | Mean      | 2.8        | 3.8          | 4.1         | 3.6  | 5.8       | 4.6  | 7.1     | 5.7         | 7.9          | 7.6          | 7.0         |
|                           | Min.      | 2.2        | 1.9          | —           | 3.1  | 4.4       | 4.5  | 4.5     | 5.7         | 7.0          | —            | 6.6         |
|                           | Max.      | 3.4        | 4.5          | —           | 4.3  | 7.4       | 4.7  | 8.4     | 5.7         | 8.9          | —            | 7.5         |
Fig. 5. Phyllosoma larvae of *Panulirus penicillatus* from Stage VII to Stage X from the western area (WP: A, C, E, and G) and the eastern area (EP: B, D, F, and H) of the Pacific Ocean. CL, cephalic shield length; AL, abdomen length; ESL, eyestalk length. Scale bar = 10 mm.
firmed the prolonged historical isolation between the western–central and eastern populations of this lobster; the EPB—the 4000- to 7000-km expanse of deep water without islands—appears to have effectively isolated the two populations (Chow et al. 2011; Abdullah et al. 2014a; Inacchei et al. 2016). Adult lobsters of the two populations on each side of the EPB have different body colors: greenish in the western–central population and reddish brown in the eastern population (George 2006, Abdullah et al. 2014a). Genetic analysis suggests that the two populations remain as subspecies of *Panulirus penicillatus* (Abdullah et al. 2014a). The discrepancies in the CW : TW values between the larvae from the WP and EP (e.g. 1.003 [WP] vs. 1.064 [EP] in the penultimate phyllosoma stage) are much larger than that between the larvae of subspecies in the *P. longipes* complex collected in the same area of the western Pacific Ocean (*P. longipes longipes* [0.852] vs. *P. longipes bispinosus* [0.852] at the same stage) (Chow et al. 2006a).

The morphological differences observed between the *P. penicillatus* larvae from the WP and EP might be genetically determined. However, environmental factors can affect larval morphology. For example, the shape of the ce-

### Table 5. Comparison of body-dimension ratios in Stage VI to X phyllosoma larvae of *Panulirus penicillatus* from the western (W), central (C), and eastern (E) areas of the Pacific Ocean. BL, body length; CL, cephalic shield length; CW, cephalic shield width; LBAW, length between the anterior margin and the widest part of the cephalic shield; TW, thorax width; AL, abdomen length; ESL, eyestalk length.

| Ratio | Stage/Area (No. of inds.) | VI | VII | VIII | IX | X |
|-------|--------------------------|----|-----|------|----|----|
|       | W (10) C (1) E (48) | W (13) C (7) E (48) | W (25) C (7) E (2) | W (22) C (1) E (3) | W (10) C (1) E (3) |
| CW : CL | 0.641 ± 0.020 0.680 ± 0.032 0.742 ± 0.018** | 0.717 ± 0.021 0.778 ± 0.012** | 0.733 ± 0.027 0.805 ± 0.022* | 0.727 ± 0.019 0.751 ± 0.031* | 0.782 ± 0.010 |
| CW : TW | 0.906 ± 0.033 0.943 ± 0.037 1.019 ± 0.023** | 0.987 ± 0.031 1.058 ± 0.009** | 1.003 ± 0.027 1.064 ± 0.010* | 0.986 ± 0.023 1.035 ± 0.039 | 1.035 ± 0.030 |
| LBAW : CL | 0.519 ± 0.026 0.502 ± 0.034 0.498 ± 0.014 | 0.485 ± 0.014 0.492 ± 0.017 | 0.481 ± 0.023 0.457 ± 0.015 | 0.495 ± 0.017 0.468 ± 0.017 | 0.453 ± 0.019 |
| AL : BL | 0.054 ± 0.005 0.058 ± 0.011 0.068 ± 0.012** | 0.103 ± 0.014 0.099 ± 0.017 | 0.155 ± 0.023 0.177 ± 0.015 | 0.246 ± 0.019 0.255 ± 0.010 | 0.259 ± 0.004 |
| ESL : CL | 0.333 ± 0.024 0.338 ± 0.013 0.332 ± 0.016** | 0.345 ± 0.019 0.321 ± 0.019 | 0.349 ± 0.019 0.310 ± 0.019 | 0.326 ± 0.019 0.321 ± 0.019 | 0.314 ± 0.015 |

**Table 6. Values of CW : CL and CW : TW reported in the literatures for *Panulirus penicillatus* phyllosoma larvae.

| Wild or cultured | The most advanced developmental stage* | Ocean | Area | CW : CL | CW : TW | References |
|------------------|---------------------------------------|-------|------|---------|---------|------------|
| Cultured         | X                                     | Pacific | West | 0.676   | 0.953   | Matsuda et al. 2006 |
| Wild             | X                                     | Pacific | West | ND      | 1.000   | Chow et al. 2006 |
| Wild             | VIII                                  | Pacific | West | 0.740   | 1.010   | Johnson 1971a |
| Wild             | IX                                    | Pacific | East | 0.812   | 1.089   | Johnson 1971b |
| Wild             | X                                     | Pacific | Center | 0.750  | 1.010   | Johnson 1968 |
| Wild             | VII                                   | Indian | South-west | 0.766  | 1.017   | Berry 1974 |
| Wild             | X                                     | Indian | Tropical and south-west | 0.746 | 0.961 | Prasad et al. 1975 |
| Wild             | VIII                                  | Indian | East | 0.732   | 1.050   | Tampi and George 1975 |

*: Staging criteria were used by Matsuda et al. (2006). ND, no data, CL, cephalic shield length; CW, cephalic shield width; TW, thorax width.
phalic shield is known to differ between wild and laboratory-produced larvae. The final phyllosomal stage (Stage X) of *P. penicillatus* cultured in the laboratory using hatched larvae from adults caught in the WP had a mean CW : TW value of 0.953 (Matsuda et al. 2006), whereas values for wild-caught larvae in the WP were 0.986 (this study) and 1.000 (Chow et al. 2006a) (Table 6). There was a similar discrepancy between wild and laboratory-produced larvae of *P. longipes bispinosus* and *P. japonicus*. The mean CW : TW values of Stages VII to X cultured larvae of *P. longipes bispinosus* were 0.784, 0.779, 0.820, and 0.813 (Matsuda and Yamakawa 2000), whereas those of wild larvae were 0.821, 0.838, 0.852, and 0.836 (Chow et al. 2006a). The mean CW : TW values of Stages VII to X cultured larvae of *P. japonicus* were 0.688, 0.697, 0.710, and 0.749 (Matsuda 2005), whereas those of wild larvae were 0.740, 0.761, 0.761, and 0.766 (Chow et al. 2006a). Mid- to late-stage wild phyllosoma larvae of *P. japonicus* also had a wider cephalic shield than cultured larvae (Hamasaki et al. 2012). Unfortunately, there are no studies of environmental factors that might affect the shape of the cephalic shield, although there must be discrepancies between the environmental conditions in a laboratory and in the wild (e.g. water current, temperature, light, and food). It will be necessary to culture phyllosoma larvae from the two areas under the same conditions to determine whether genetic or environmental factors induce the difference in the shape of the cephalic shield.

Several publications have described *P. penicillatus* phyllosoma larvae collected in the ocean. In the Pacific Ocean, Johnson (1971a) reported a Stage VIII phyllosoma larva (21-mm BL) caught in the South China Sea (Table 6). The body shape appears to correspond to the body shape of the larvae from the WP in the present study. Moreover, a Stage X larva (32-mm BL), equivalent to our Stage IX, collected to the east of the EPB off the coast of Mexico, was described and illustrated by Johnson (1971b). This larva had a body shape similar to those of the larvae from the EP in the present study.

Johnson (1968) caught 128 larvae of this species in Hawaiian and neighboring waters (CP) and described some developmental stages. His later-stage larvae have CW : CL values of 0.80 at our stage VIII, 0.78 at our stage IX, and 0.75 at our stage X, indicating that the body shape of his specimens resemble that of the larvae from the EP in the present study. In the present study, we only collected two *P. penicillatus* larvae from the waters near the Hawaiian archipelago; they tended to have morphologies similar to those from the WP. These discrepancies between the larvae of Johnson (1968) and this study suggest that larvae in the CP vary widely in the body shape.

There are also some reports of *P. penicillatus* phyllosoma larvae from the Indian Ocean. A phyllosoma larva (19.8-mm BL, our Stage VII) caught off the Natal coast of South Africa had CW : CL and CW : TW ratios of 0.766 and 1.017, respectively, seemingly closer to the body shape of the larvae from the EP than of those from the WP (Berry 1974) (Table 6). Prasad et al. (1975) roughly illustrated a sequence of developmental phyllosoma stages from Stage VII to Stage XII from the tropical and southwestern areas of the Indian Ocean. Their Stage XII, corresponding to our Stage X, has CW : CL and CW : TW ratios of 0.746 and 0.961, respectively, indicating that their CW : CL ratio is intermediate between those of the larvae from the WP and EP in the present study and their CW : TW ratio is close to the ratios of the larvae from the WP. Tampi and George (1975) also provided a sketch of a Stage IX larva (25.5-mm BL, equivalent to our stage VIII) from the eastern Indian Ocean; the larva has CW : CL and CW : TW values of 0.732 and 1.050, respectively, and the body shape appears to have characteristics of both the larvae from the WP and EP in the present study.
study. Abdullah et al. (2014b) observed distinct genetic isolation of populations at the northwestern and southwestern edges of the distribution of *P. penicillatus* in the Indian Ocean, and gene flow within the population in the central and eastern regions of the ocean. Therefore, there is probably a wide range of variation in the larval morphology of *P. penicillatus* in the Indian Ocean, and the morphology of some larvae within this range of variation may differ from morphologies of WP and EP larvae in the Pacific Ocean.

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